THE ANOPHELINES OF INDIA

(REVISED EDITION)

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MALARIA RESEARCH CENTRE
INDIAN COUNCIL OF MEDICAL RESEARCH
1984

Price: Rs. 150.00
U.S.$ 45.00

Published by the Malaria Research Centre (ICMR) 22, Sham Nath Marg, Delhi-110054 and Printed at Delhi Press, 3E, Jhandewalan, New Delhi-110055

DEDICATED

TO

All my **Fellow Workers** who gave their best to conquer malaria but found to their disappointment that the enemy had outwitted them. Let better results attend the efforts of those who follow us fortified by an intimate knowledge of the enemy and his strength and weaknesses.

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CONTENTS

FOREWORD

The waxing and waning saga of malaria is contemporary history. The hopes that malaria would be 'eradicated' following the flush of initial successes with DDT have been belied. The future of malaria control has elements of doubts and uncertainties. The biological front is never static; as newer insecticides are discovered vector resistance is likely to manifest. It is human ingenuity alone that has a chance of overtaking biological evolution.

The biological factors of vector and parasite resistance are not the only difficulties in our way; the human factors and failures have also played a major role. Following early success with DDT and the tumbling down of malaria mortaility and morbidity, there was little interest in malariology and original minds had no attraction to study either the vector or the parasites, or the clinical disease which they produce. The whole edifice of malaria research and malaria surveillance crumbled. Sustained studies on vectors and parasites of malaria were few and far between. Mankind was caught napping and malaria resurgence took the third world by storm. The price of health is eternal vigilence. Malaria control has been compared to a guerrilla war. Any relaxation of control measures will lead to the activation of the guerrillas.

The World Health Organisation has taken the initiative in mobilising world resources for a new intensive study of malariology in order to develop newer methods of malaria control based on modern cell biology and molecular biology. To activate research in this field and to build adequate scientific man power for research and for surveillance, the Indian Council of Medical Research has embarked upon an extensive programme of research on malaria in all its aspects such as epidemiology, parasitology, vector biology, chemotherapy, immunology, and operational research for its control. There is no alternative to continuing systematic studies of these aspects of malaria if we are to mount an intelligent attack on malaria. Knowledge of the habits and habitats of the vectors responsible for transmission of malaria in different ecological settings in the country is a *sine quanon* for the successful pursuit of any control programme. It is upon an adequate understanding of vector biology and ecology in various geographic regions of the country that a sound programme of control measures can be instituted. The strategies to be used need to be adapted to the local species and their behavioural characteristics. A single formula derived at the Centre irrespective of these regional variations, will not do.

At this critical juncture, the Indian Council of Medical Research is happy to publish this monumental work on "The Anophelines of India" by Dr. T. Ramachandra Rao. Such a volume is urgently needed so that the malaria workers have in their hands an up to date, authoritative and comprehensive publication which brings together all that is known about distribution, bionomics, ecology, relation to

disease and basic principles of control. It represents the life work and mission of Dr. Rao who has spent his entire life in the study of mosquitoes, in the transmission of malaria in the early stages of his career and later on their role as vectors of virus diseases. The Indian Council of Medical Research is indeed fortunate to have him write this book. There are few in the world today who can equal Dr. Rao in the depth of knowledge, understanding and scholarship in this area, so vital to the future of mankind.

Dr. Rao has approached this onerous task in a unique way. The emphasis is on bionomics and ecology but the account given is more comphrehensive and holistic and with a high degree of objectivity. There is not only a bird's eye view of the whole subject but also a detailed perspective on each species. The chapter on "Land-marks in control of anophelines as a means to the control of malaria" has historic significance from where one can learn many lessons today. The results of all major studies carried out in the last 45 years have been incorporated. The result is a book which is eminently readable and is intensely practical. It should help not only the worker in the field in his daily tasks but also stimulate the researcher to devote himself to this fascinating area.

One final comment: Mosquitoes are of great interest to students of biology in general. Mosquito bionomics can be a fascinating model for exemplifying many types of animal behaviour and ecology and the book is replete with references to the relationship of the mosquito to the environment. There are many aspects of mosquito biology on which students of Zoology in the universities can profitably undertake research.

It is a privilege for me to introduce this monograph to the scientific community as a contribution of the ICMR towards the study of vector biology as a fascinating field in its own right and towards a more scientific appraoch to the control of malaria and other vector-borne diseases in our country.

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June 23, 1980

PREFACE TO THE REVISED EDITION

This revised edition brings out the available information on Indian Anophelines up to date, and also includes all textual and typographical corrections found necessary. The author once again thanks Prof. V. Ramalingaswamy, Director General and Dr. G.V. Satyavati, Editor in Chief, Indian Council of Medical Research New Delhi for their unstinted support and encouragement. To Dr. V.P. Sharma, Director, Malaria Research Centre (ICMR), Delhi, are due special thanks for the immense trouble he has taken to bring the edition out and for many constructive suggestions.

I am greatly indebted to the staff members of publication department and several scientists of Malaria Research Centre for having made invaluable efforts in bringing out this revised edition, in particular, Dr. V.K. Gupta, Dr. P.K. Srivastava, Mr. Tushar K. Varma, Mr. B.N. Nagpal, Mr. V.R.T. Nair, Mr. Chander Mohan and Miss Sujata Sett.

Thanks are due to Mr. P.N. Ranga Rao, former Entomologist, Malaria Organization, Maharashtra, for his painstaking effort in checking the text page by page. Personal thanks are also due to several other friends who have helped in many ways.

The management of Delhi Press, Delhi, especially Mr. Prakash Pant and Mr. Ravindra Nath Pandey who have been very courteous and cooperative, deserve many thanks.

October, 1983

T. RAMACHANDRA RAO

PREFACE TO THE FIRST EDITION

This monograph has been prepared with the object of providing malaria workers in India with accurate and up to date information on the distribution, ecology, relation to disease and control of the anophelines of India. Previously there were two excellent publications, one by Sir Rickard Christophers in 1933 on "Anophelini" in the "Fauna of British India" Series, and the other in 1961 by several authors in a book entitled "The Vectors of Malaria in India" (second edition) published by the National Society of India for Malaria and other Mosquito-borne diseases. Christophers' book was mainly a taxonomical monograph but also contained brief but valuable information on many aspects of *Anopheles* biology. The second book dealt with ecology, relation to disease and control of only the ten known vectors of malaria. Unfortunately both of them have become rather outdated. Therefore, a comprehensive book dealing with all taxa of *Anopheles* of this country and the neighbouring regions had long been overdue.

In view of the resurgence of malaria, it is essential that the new band of malaria workers who are to come up to shoulder the responsibility of *Anopheles* control should have a good knowledge of the biology of the anopheline species and should have a source book of information on the research done on them. Further, in view of the re-emphasis being placed on control measures of various types best suited to each species and locality to replace the single countrywide practice of indoor residual spryaing by insecticides prevailing during the last two decades, workers should have a thorough knowledge of the ecology and behaviour of the vectors, in addition to a good knowledge of the history of *Anopheles* control in this country. It is hoped that this monograph will serve these purposes.

Further it has to be pointed out that so far as taxonomy is concerned, the present author has not felt equal to the task of revising the classic volume by Christophers and therefore detailed and complete descriptions of the morphology and chaetotaxy of the individual species have not been attempted. However, as a basic knowledge of taxonomy is important for proper recognition of the vector species and its variants, all major taxonomical advances since the publication of Christopers' book have been briefly incorporated.

The question of including keys for the identification of anophelines was carefully considered and it was decided to include them. Keys for all stages based on the excellent keys provided by Christophers have been included with modifications necessitated as a result of recent work on the taxonomy of some major groups of anophelines. Grateful acknowledgment in this connection is made to the Director, Zoological Survey of India, Calcutta, for his permission to utilise Christophers' keys.

Regarding distribution, bionomics, ecology and relation to disease, the results of all the major studies carried out during the last 45 years have been included in respect of every species. In order to keep the book within a reasonable size, greater emphasis had to be placed on the more significant and sustained studies rather than on several casual reports of a merely confirmatory nature. The bibliography will be found to contain practically all important papers published since 1933. For the period prior to that year only very important publications of a historical nature have been included. From this point of view, it can be assumed that the present work takes off from the point where Christophers left it.

A comment is necessary with regard to the information on the results of dissections for the purpose of incriminating the vector. Detailed and analytical statements regarding the vectorial status of each species have been made in a narrative form and it has been decided not to attempt to publish complete lists of the dissection work carried out by various workers, as was admirably done by Covell (1927), Horsfall (1958, Reprinted 1972) and by several authors in the book on "The Malaria Vectors of India" in 1961.

The history of *Anopheles* control in India is replete with many outstanding contributions both to the theory and practice of mosquito control. A complete account of them would require a volume by itself. Therefore, only selected examples of *Anopheles* control which have significantly added to practical malariology have been mentioned in the chapter on "Landmarks on control of anophelines as a means to the control of malaria."

During the last 27 years, the public health workers of this country have been busy with single-minded devotion to combat malaria, first in a five year National Malaria Control Programme and during the next 22 years on a National Malaria Eradication Programme. Most of the field workers, unfortunately, were not specialist malariologists or specialist entomologists, but mainly trained to carry out the prescribed operations. They did their work very well but they had little opportunity to pay that degree of attention to research which was needed. Many of their observations are locked away in unpublished official records and only a few have appeared in print. Most of the studies have, however, been mainly of a routine nature on the effect of the insecticide treatment on densities of the adults of the vectors, on insecticide suseptibilty, and occasionally on age determination. Not many of these studies have been of a critical nature; therefore, only reliable information on the biology of anophelines has been taken into consideration.

When dealing with ecology and relation to disease of several species occuring in India, much information gathered on them in neighbouring countries has been used. Such information is extremely important to us, the Indian entomologists, for understanding the behaviour of the species within their own country and will help them to interpret their findings in the light of knowledge gathered over a wider horizon. Though behaviour of mosquitoes may vary somewhat from one geographical region to another, they are not respecters of political boundaries. There is much

in common in the behaviour of many of the species all over South and Southeast Asia.

Apart from the general chapters dealing with such subjects as distribution, biology, relationship to disease and control, there is a special chapter dealing with individual species in which these subjects, along with taxonomical notes, have been discussed more extensively. Therefore, not only a bird's eye view of the whole subject but also a detailed perspective on each species has been provided.

Workers in different regions of India would need to have information on the previous studies made in their respective areas. Instead of merely giving distribution lists of anophelines district by district, as was done by several previous authors, a special chapter on surveys in the nine recognized zones of the country has been prepared, including in it the lists of species detected and a brief account of the malaria vectors found. Only the major surveys carried out and reported have been included. In addition, a chapter on the anophelines of neighbouring countries has been compiled.

A chapter on Speciation, Genetics and Cytotaxonomy has been included to acquaint the workers with the great advances made in these modern subjects during recent years with special reference to anophelines. They are going to play an important part not only in taxonomy, but also in the understanding of the behaviour, disease susceptibilities, the effectiveness of the insecticides and newer method of control.

The general objectives of this book has been to provide a balanced and comprehensive account of the anophelines of India in all aspects, with emphasis on bionomics and ecology. Every effort has been made to keep a high sense of objectivity. It is hoped that a subjective bias, if any, understandable in a person who has been intimately studying Indian anophelines for over four decades, has not crept in.

A wide search of literature published during the last five decades has been made and over 1,000 articles, books and other references have been seen personally, not only of publications on Indian anopheline fauna but also of many papers published on fauna of neighbouring countries. Because of compulsions of space and the set objectives, some selection has been made in the preparation of the bibliography to restrict it to the absolutely essential ones. As far as possible reliance has been placed on published material only but in a few rather negligible instances some hitherto unpublished significant information within the author's knowlege has been included.

Considerable thought was given to whether the entomological techniques connected with anophelines should be described. There are excellent publications which deal with the subject very thoroughly. Therefore, it was decided not to include the descriptions of techniques, but merely to draw the attention of the workers to the appropriate publications.

No great prophet is required to tell us that, as we see today, malaria, filariasis and certain mosquito-borne virus diseases will be with us for some time to come.

Sound knowledge about their vectors is the foundation on which we can hope to control them effectively. It was unfortunate that during the last two or three decades there was stagnation in research on this subject, but it is hoped that with the tendency to develop newer approaches to deal with these diseases greater emphasis will be laid on studies on the habits and habitats of the vectors. This humble effort will not go in vain if this book will become a source of information and encouragement to young workers who are now sharpening their weapons to meet the challenge posed by insect vectors of diseases.

Finally, the views and opinions, if any, expressed in this book are those of the author and should not be taken necessarily to reflect those of the Indian Council of Medical Research.

June 1980

T. RAMACHANDRA RAO

ACKNOWLEDGEMENTS

I am highly indebted to Dr. C. Gopalan and Prof. V. Ramalingaswami, the successive Directors-General of the Indian Council of Medical Research during the period of preparation of the monograph, for their unstinted encouragement and material and moral support. The idea of preparing such a monograph had been in my mind for several years, but it had remained unfulfilled because I was aware of the enormity of the work involved which needed full time attention. The successful completion of this monograph was possible only because of the continued and enthusiastic support from both Dr. Gopalan and Prof. Ramalingaswami. They have appreciated the urgent need for a book such as this in the present context of the expanded research programme on malaria which the Council has embarked upon. My thanks to them and to the Council for financial support cannot be adequately expressed.

Dr. V.N. Rao and Dr. N.P. Gupta, former Senior Deputy Directors-General of the Indian Council of Medical Research in charge of Epidemiology and Communicable Diseases have taken keen personal interest in the project and have continuously given their whole-hearted support. It was Dr. Gupta's personal encouragement and assurance of full cooperation from the National Institute of Virology, Pune, when he was its Director, helped me in taking the final decision to undertake this work. Their positive and sympathetic approach in preparation of this monograph has been a major factor in its successful completion.

Mr. K.S. Krishnan, former Assistant Director (Entomology) of the National Malaria Eradication Programme and an erstwhile colleague of mine, has helped me in the actual work of preparation of this monograph. He has helped me in revivewing the manuscript as it was developing, in the preparation of the bibliography and by wise counsel on several occasions. He deserves my sincere thanks.

Special acknowledgement of the valuable role played by Dr. G.V. Satyavati, Deputy Director General, Publications and Information, I.C.M.R., New Delhi, has to be made for sustained interest and advice. It was her special interest which made the publication work smooth and timely

While I have received assistance from a large number of fellow workers in one way or another and they are too numerous to mention, I would be failing in my duty if I did not specifically mention the following: Dr. S. Pattanayak, Dr. R.G. Roy, Mr. B.S. Krishnamurthy, Dr. C. Achuthan, Dr. G. Raman and Mr. C.P. Vijayan of the National Malaria Eradication Programme; Dr. B.L. Wattal, Mr. N.S. Kalra, Mr. R. Rajagopal and the late Mr. B.N. Mohan of the National Institute of Communicable Diseases; Dr. K.M. Pavri, Dr. V. Dhanda and Dr. H.R. Bhat of the National Institute of Virology, Pune; Dr. P.K. Rajagopalan and Dr. R. Reuben of the Vector Control Research Centre, Pondicherry; and Dr. V.P. Sharma and Dr. Sarala Subba Rao of the Malaria Research Centre, (I.C.M.R.) Delhi. They

have helped me in several ways.

I have used the facilities of the following libraries to the authorities of which my thanks are due: Indian Council of Medical Research (Librarian: Mr. S.M. Ahmed), National Institute of Communicable Diseases, Delhi; National Institute of Virology, Pune (Librarians: Miss I. Navathe and Miss F. Watchmaker); Vector Control Research Centre, Pondicherry; Pasteur Institute, Coonoor; National Medical Library, New Delhi; The Indian Institute of Science, Bangalore and Regional Co-ordinating Organisation, N.M.E.P., Bangalore. Mr. Ahmed has been of very valuable help in tracing old literature.

Valuable reprints and publications have been received from: Dr. A. Stone, Dr. B.A. Harrison, Dr. J.B. Kitzmiller of U.S.A., Dr. R. Pal and Dr. R. Darwish of WHO., Prof. M. Aslamkhan of Pakistan, Mr. M.S. Shrestha of Nepal, Dr. B.N. Chowdaiah, Dr. N.L. Sitaraman and Dr. C. Achuthan of Bangalore, and several others.

Permission to reproduce brief excerpts from certain publications have been obtained from authors, particularly Dr. G.B. White and Dr. N.H. Reid.

I am indebted to several scientists for having given me authoritative information on the types deposited in the different museums and institutions. They are: Dr. G.B. White, British Museum, London; Dr. H. Schumann, Museum for Naturkunde, der Humboldt Universitat, Berlin, DDR; Dr. P.J. van Helsdingen, Naturhistorisches Museum, Vienna; Dr. Wayne N. Mathis, National Museum of National History, Smithsonian Institution, Washington; Dr. Loic Matile, Museum National D'Histoire Naturelle, Paris; Dr. B.L. Wattal, National Institute of Communicable Diseases, Delhi and Prof. T.N. Ananthakrishnan, Zoological Survey of India, Calcutta.

The stenographic work and typing assistance by Mr. P. Sundarajan, Mr. Ananthanarayana, and Mr. V. Rajagopal and art work Mr. M.A. Potnis and Mr. H.M. Shinde of the National Institute of Virology, Pune, are acknowledged with pleasure.

Miss Geetha Bai of VCRC, Pondicherry deserves my thanks for her help in the preparation of index.

My daughters, Uma and Vasanti, and grandson, Shridhar, have been of much help in the mechanics of handling a manuscript of this size.

Finally, the authorities of W.Q. Judge Press, Bangalore, who have been uniformly courteous and cooperative, deserve my thanks.

February 1981 T. RAMACHANDRA RAO

PART I

GENERAL SECTION

1

The Name

The name *Anopheles* was first applied to a mosquito in 1818 when Meigen, a German taxonomist, renamed *Culex claviger* Meigen, 1804 as *Anopheles claviger*. The type locality was in Germany. At the same time he also named *Anopheles maculipennis*, also from Germany. The type specimen of *A. claviger* is stated to be non-existent and therefore according to a resolution of the International Committee on Zoological Nomenclature, *A. maculipennis* was designated in 1959 as a logotype (Knight and Stone, 1977). *A. claviger* has a wide distribution in Europe, North Africa, West Africa, Arabian Peninsula, Iran, Afghanistan and Khazak USSR. *A. maculipennis* also has a more or less similar distribution. Neither of them occurs in India.

The origin of the word "Anopheles", is interesting. According to Webster's International Dictionary (Third Edition, 1966) it is derived from Greek and means "hurtful" or "harmful" (*An* = not; *opheles* = fruitful). The Greek word itself has originated from Sanskrit. *Opheles* is derived from *ā phalam*, i.e., *ā* towards; *phalam* = fruit; therefore fruitful.

The members of the genus *Anopheles* have been variously known in English as anophelines, anopheline mosquitoes and often merely as malaria mosquitoes. The last name is appropriate if not quite accurate, because all known vectors of human malaria are anophelines, though all anopheline species are not vectors.

There does not seem to be any name especially applicable to anopheline mosquitoes in any of the Indian languages. They are included under the general name of mosquitoes:

Mashā, Mashakah (Pl: Mashakāh) (Sanskrit), Mashā, Mashak (Assamese), Mashā. Mashak (Bengali), Dās (Gujarati), Machchar (Hindi), Solle, (Kannada), Moh (Kashmiri), Kothuku (Malayalam), Dās (Marathi, Mashā, Mashak (Oriya), Machhad (Punjabi), Koshu, Kochu (Tamil), Doma (Telugu), Machchad (Urdu).

Mosquitoes have been known in India from very ancient times. Though not known as vectors of malaria, a disease which however had been described, they were known as a cause both of nuisance and human disease.

Russell (1955), in his studies of historical references to malaria and mosquitoes, has quoted a very interesting article by one Sir Henry A. Blake, Governor of Ceylon, read before the Ceylon Branch of the British Medical Association on April 15, 1905. Blake got several verses from 'Susruta Samhita', a Sanskrit work of ancient India, translated. There were twelve kinds of life destroying and terrific insects.

"Their bite is as painful as that of serpents, and causes diseases resulting from the three humours joined together; the bite as if burnt with caustic or fire, is red, yellow, white and pink colour accompanied by fever, pain of limbs, hair standing on end, pain, vomiting, diarrhoea, thirst, heat, giddiness, yawning, shivering, hiccup, burning sensation, intense cold, vesicles or pustules increasing, swellings, knots under the skin, circles, etc."

Susruta* named five kinds of mosquitoes (*Mashakah*) viz:

Samudrāh (marine or coastal mosquito), *Parimandalah* (very small mosquito), *Hastimashakah* (elephant mosquito, perhaps because of large size), *Krishnah* (black or dark mosquito), *Pārwatiyah* (hill mosquito). The names are all in the masculine gender.

Their bite cause "swelling on the part bitten, itching. As for the *Parwatiyah* it has similar qualities to life taking insects." Obviously our ancients had attempted classification of insects and mosquitoes and had recognised their role in the causation of human pain and disease.

Does *Parwatiyah* refer to the foothill species such as *A. fluviatilis* and *A. minimus*?

The author of the *Samhita* (compendium or treatise) was Susruta the first who lived in the fifth century B.C. His work was named *Salyatantra* and was mainly concerned with surgical matters. Another Susruta living in the second century A.D. wrote (anonymously it appears) another *Susruta Samhita* revising and complementing the original work of Susruta the first and added much new material. Perhaps the second Susruta was the great Nagarjuna himself of the second century A.D. (Kutumbiah, 1969). Therefore knowledge of mosquitoes and their relationship to disease is at least 1800, if not 2400, years old in India.**

Mosquitoes, including anophelines, have been known for a long time also in the West as well as in China. Russell (*loc.cit.*) has given a comprehensive account of the subject. Linneaus (1758 *et seq.*) had named two mosquitoes which are known today as true mosquitoes and have valid names, viz: *Culex pipiens* L. 1758 and *Aedes aegypti* (L), 1762.

The latter had actually been named *Culex aegypti* by Linneaus.

The recognition of the anopheline species and naming them scientifically in India soon followed the discoveries by Ross in India in 1898 and by Grassi and colleagues in Italy in 1899 of the role which they play in the transmission of malaria. Only three of the species of *Anopheles*, now known to occur in this country, had been named earlier, viz., *Anopheles (hyrcanus) sinensis* by Wiedmann in 1826 from a

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*The present author has re-examined the text of *Susruta Samhita* as published by the University of Mysore, with the original Sanskrit text and translation into Kannada by Bhattacharya (1973) and has confirmed the accuracy of the translation.

**The meaning of 'Parimandalah' has been further examined. According to the eminent Sanskrit scholar of Bombay, Pandit Mahuli Gopalacharya, it means 'smallest of the small'. The Monier-Williams Sanskrit-English Dictionary. Clarendon Press, (1964 Edition) gives the following meanings:—round, circular, globular; of the measure of an atom; a species of venomous gnat; globe, sphere, orbit, circumference, etc. The exact meaning in the present context is not clear. Probably, it means a very small mosquito.

locality in China, and *A. barbirostris* and *A. annularis* both by Van der Wulp in 1884 from Indonesia. If the record of *A. vanus* Walker, 1860, by Wattal *et al.* (1962) is further confirmed by taxonomists, it would be another species named earlier to Ross's discoveries.

The first series of papers dealing with the taxonomy of Indian anophelines commenced with that by Grassi (*A. subpictus*) (1889) and was followed by those of Giles (1901), Theobald (1901-1902), Donitz (1901), Liston (1901), James (1902), Cogill (1903), James and Liston (1904-1911) and others. The species of *Anopheles* in which Ross in 1897 had found oocysts in Secunderabad (dapple winged mosquito) was probably *A. stephensi* named by Liston in 1901. Several other workers followed in the ensuing three decades adding many new species names of *Anopheles* in India and neighbouring countries and new knowledge about their distribution and ecology. In 1932, Edwards published his famous volume on mosquitoes in the 'Genera Insectorum'. The larvae of Indian anophelines were studied intensely by Puri resulting in his comprehensive monograph on the subject (Puri, 1931). The culmination of all this work was the publication of the classic volume on "Anophelini" by Christophers (1933) in the Fauna of British India, published under the authority of the then Secretary of State for India. The volume not only included extensive information on the names and systematics of the then known 42 species and 10 varieties of anophelines, towards an understanding of which Christophers himself had contributed much, but also included brief but useful notes on the breeding habits, adult bionomics, distribution and relation to disease.

Since 1933, very few names have actually been added but several changes in the taxonomical status of some species have taken place. Among the new names which have come into use is *A. stephensi* var. *mysorensis*. Some species which occur in India but had not been previously recognised are, *A. crawfordi*, *A. roperi* and *A. argyropus*. A new name, *A. nitidus*, has been added, *A. leucosphyrus* has been taken out of the list of Indian anophelines, but it has been shown that the species of this group occurring in India are *A. balabacensis* and *A. elegans*. The studies on *hyrcanus* and *barbirostris* groups have also led to several changes. *A. hyracanus sensu* Pallas does not seem to occur in India and the main variety in India has been raised to the status of a full species viz., *A. nigerrimus*. *A. peditaeniatus* has been raised from a synonym of *A. nigerrimus* to a full species status and *A. indiensis* has become a synonym *A. barbumbrosus* and *A. vanus*(?) of the *barbirostris* group are now reported from India. Changes in the taxonomic status of a few varieties, subspecies and species have occurred. For example, *A. aitkenii* var. *bengalensis*, *A. barbirostris* var. *ahomi* and *A. annandalei* var. *interruptus* have now been raised to the full species status. A few species formerly included in the fauna of undivided India have now been removed because they are known to occur in Pakistan only. They are *A. superpictus*, *A. sergenti* and *A. habibi*. Many of these changes and additions are the result of intensive work on taxonomy carried out in Southeast Asian countries. In the present work the names as listed by Knight and Stone (1977) in the latest edition of the *Catalog of the Mosquitoes of the World* have been followed except where otherwise indicated.

2

Systematics: Classification

Genus Anopheles belongs to *Class*: Insecta, *Order*: Diptera, *Suborder*: Nematocera and *Family*: Culicidae.

The order Diptera are described very briefly as follows:

"Insects with a single pair of membranous wings, the hind pair modified into halteres. Mouth parts suctorial, usually forming a proboscis and sometimes adapted for piercing; labium usually distally expanded into a pair of fleshy lobes. Prothorax and metathorax small and fused with the large mesothorax; tarsi commonly 5 jointed. Metamorphosis complete; larvae eruciform and apodous, frequently with the head reduced. Tracheal system variable, most often amphipneustic. Pupa either free or enclosed in the hardened larval cuticle or puparium. Wing tracheation reduced." (Imms, 1938).

Diptera is one of the largest orders of insects and includes many flies of medical importance and structurally most highly developed among insects.

The suborder *Nematocera* can be described as follows:

"Antennae of imago many jointed, longer than the head and thorax; the majority of the joints alike; arista wanting. Palpi usually 4-5 jointed, pendulous. Discal cell (of wings) generally absent, cubital cell when present widely open."

Larvae with a well developed exserted head and horizontally biting mandibles; pupa free. In mosquitoes, however, the palpi are stiff and not pendulous but projecting. The suborder includes many families such as *Psychodidae* (Sand flies), *Simuliidae* (black flies) and *Chironomidae* (midges — Culicoides) and *Culicidae* (mosquitoes) all of great medical importance.

Family Culicidae (mosquitoes) can be described briefly as follows:

"Very slender flies, generally with an elongated piercing proboscis and no ocelli; the palpi stiff and not pendulous, legs long, antennae densely plumose in the males, pilose in the females. Wings fringed with scales along the posterior margin and the veins. Larvae and pupae aquatic and very active, the former metapneustic, with an enlarged thoracic mass" (Imms, *loc. cit.*).

In the modern classification of Culicidae as adopted by Knight and Stone (1977) there are three subfamilies, viz:

Subfamily *Culicinae* with thirty genera.
Subfamily *Anophelinae* with three genera viz.,
Chagasia, Bironella and *Anopheles:*
Subfamily *Toxorhynchitinae* with one genus, viz.
Toxorhynchites.

Subfamily Culicinae

According to Stone *et al.* (1959) there were 27 genera placed in two tribes, viz. *Sabethini* and *Culicini*, under Culicinae.

However, in the revised edition of *The Catalog of the Mosquitoes of the World*, Knight and Stone (1977) (updated to 1973) have included 30 genera under ten tribes as follows:

Tribes	*Genus*
1. Aedeomyiini	*Aedomyia*
2. Aedini	*Aedes, Armigeres, Eretmopodites, Haemagogus, Heizmannia, Opifex, Psorophora, Udaya*
3. Culicini	*Zeugnomyia, Culex, Dinoćerites, Galindomyia.*
4. Culisetini	*Culiseta.*
5. Ficalbiini	*Ficalbia, Mimomyia.*
6. Hodgesiini	*Hodgsesia.*
7. Mansoniini	*Coquellettidia, Mansonia.*
8. Orthopodomyiini	*Orthopodomyia.*
9. Sabethini	*Limatus, Malaya, Maorigoeldia, Phoniomyia, Sabethes, Topomyia, Trichoprosopon, Tripteroides, Wyeomyia.*
10. Uranotaeniini	*Uranotaenia.*

In the naming and classification of tribes under *Culicinae* Knight and Stone have followed Belkin (1962). Three new genera have been added: *Galindomyia, Maorigoeldia* and *Mimomyia*. So far as Indian Culicinae are concerned, Barraud's *Fauna of India* volume (1934) still holds its place as the most comprehensive monograph, though some work on taxonomy has been done since then.

Subfamily Anophelinae

The genus *Chagasia* has four species all occurring in the New World, that too in the countries south of the United States of America, extending from Mexico to Argentina and some neighbouring localities.

The genus *Bironella* has two subgenera, viz. *Bironella* and *Brugella*. Subgenus *Bironella* has five species, viz. *bironelli, brugi, confusa, papuae* and *soesiloi*. Their range of distribution is from New Guinea (Present names: West Irian and Australian New Guinea) to Australia. Subgenus *Brugella* has two species, *hollando* and *travestita*, occurring in the New Guinea and Bismark Archepelago areas. Neither of the above two genera has been recorded in India.

The separation of the genera *Chagasia* and *Bironella*, which do not occur in India, from *Anopheles* is based on a few key points, viz. (adopted from Christophers, 1933):

	Chagasia	*Bironella*	*Anopheles*
Adult			
Tarsal claws in male	2 large claws on II and III legs	Single claw on fore leg and without a median or basal spur.	Single claw on fore leg with a median and basal spur except in *A. culiciformis* which has no basal spur.
Scutellum	Slightly trilobed with a set of bristles on each lobe	Bar-shaped or evenly rounded with continuous line of bristles.	Bar-shaped or evenly rounded with continuous line of bristles.
Claw of fore leg of male	—	Without median or basal spur	With median or basal spur
Vein 5.1	—	Concave beyond cross vein.	Not concave beyond cross vein.
Larva			
Lateral borders of scoop	Bearing fringe of hairs	Without fringe of hairs.	Without fringe of hairs.

(The differences between *Anopheles* and the common culicines will be dealt with later.)

Genus Anopheles

Unlike Christophers (1933), who had recognised four subgenera, viz. *Stethomyia, Anopheles, Nyssorhynchus* and *Myzomyia*, Knight and Stone (1977), now accept six subgenera, viz. *Stethomyia, Anopheles, Nyssorhynchus, Kerteszia, Lophopodomyia* and *Cellia*. The main differences are: (1) acceptance of separate subgenera *Kerteszia* Theobald including forms other than those included under *Nyssorhynchus* by Christophers and *Lophopodomyia* Antunnes; and (2) all members of the subgenus *Myzomyia* are now placed under the subgenus *Cellia*.

There are in all about 365 recognized species of *Anopheles* in the world of which 149 belong to subgenus *Anopheles*, 172 to *Cellia*, 9 to *Kerteszia*, 6 to *Lophopodomyia*, 24 to *Nyssorhynchus* and 5 to *Stethomyia*. Fifty-one species and 7 subspecies and varieties belonging only to subgenera *Anopheles* and *Cellia* occur in India.

According to currently accepted classification, there are six subgenera of *Anopheles*, viz:

Stethomyia Theobald, 1902
Kerteszia Theobald, 1905
Nyssorhynchus Blanchard, 1903
Lophopodomyia Antunnes, 1937
Anopheles Meigen, 1818
Cellia Theobald, 1902

As Reid (1968) has stated, it is difficult to construct a perfect key to the subgenera. The separation of the subgenera is mainly made on the basis of number and position of spines on the coxites of the male genitalia. Geography-wise, the subgenera can be classified as follows:

Exclusively New World subgenera

Stethomyia, Kerteszia, Nyssorhynchus, Lophopodomyia

Present both in the New World and the Old World

Anopheles

Exclusively Old World species.

Cellia (= *Myzomyia sensu* Christophers)

As we are concerned only with subgenera *Anopheles* and *Cellia*, only their differences will be dealt with here. For other subgenera Christophers (1933), Reid (1968) and other taxonomical works may be seen.

Some characters distinguishing subgenera *Anopheles* and *Cellia* are:

	Subgenus Anopheles	*Subgenus Cellia*
Adult		
Wing:	Entirely dark or with less than 4 dark areas involving both costa and vein 1.	With at least 4 dark areas on costa involving both costa and vein 1.
Parabasal spines on male coxite:	Two plus one internal spine.	4 or 5 and no internal spine.
Female cibarium:	Without teeth	With teeth.
Larva		
Antennal hair:	Usually branched (except in tree hole species) located on inner or dorsal side of shaft.	Usually simple located on the outer side of the shaft.
Inner clypeal hair:	Close together.	Wide apart.
Long pleural hairs on thorax:	Usually simple.	Some at least usually branched.
Pupa		
Male genital pouch:	Without 2 knobs or points.	With 2 knobs or points.
Terminal hair on paddle:	Rarely long and hooked.	Usually long and hooked.

Subgenus *Anopheles* Meigen

The subgenus *Anopheles* has been classified by Reid and Knight (1961) as follows:

Laticorn section .. *Myzorhynchus* series

Angusticorn section .. *Anopheles* series, *Lophoscelomyia* series.

Three other series have been recognized, viz:

Cycloleppteron New World

Arribalzzagia New World

Christiya Ethiopian region

The two sections have been proposed mainly on the characters of the pupae. For details, reference may be made to Reid and Knight (1961). For the use of students of Indian anophelines, a simpler key would be as follows:

1. Apex of hind femur with a prominent tuft of black and white scales; front femur somewhat swollen at base; *Leophoscelomyia*
   Apex of hind femur without a prominent tuft of scales.
2. Wing scales all dark without pale scales. *Anopheles* (part)
   Wings with some pale scales but not more than three dark areas involving costa and vein 1. 3
3. Front femora markedly swollen at base; female palpi shaggy *Myzorhynchus*

   Front femora slender or only indistinctly swollen at base; scales of female palpi appressed; palpi not shaggy. *Anopheles* (part)

The members of the three series found in India are:

Anopheles	*aitkenii* group	*aitkenii, bengalensis*
	culiciformis group	*barianensis, culiciformis, pinjaurensis, sintoni*
	lindesayi group	*lindesayi, lindesayi* ssp *nilgiricus, gigas* and its varieties.
Lophoscelomyia	*asiaticus* group	*annandelei, interruptus*
Myzorhynchus	*hyrcanus* group	*argyropus, crawfordi, nitidus, nigerrimus, peditaeniatus, sinensis*
	barbirostris group	*ahomi, barbirostris, barbumbrosus, vanus (?)*
	umbrosus group	*roperi, umbrosus*

Subgenus *Cellia* Theobald

The mosquitoes of the subgenus *Cellia* are grouped into six series:

Neomyzomyia	Ethiopian, Oriental and Australian regions
Pseudomyzomyia (=Pyretophorus);	Oriental and Ethiopian regions
Paramyzomyia	North Western Indian, West Asian, East African, West African and Mediterranean regions
Myzomyia	Ethiopian, Mediterranean and Oriental regions
Neocellia	Oriental and some in Ethiopian regions
Cellia	All Ethiopian

The group *Cellia* does not occur in India.

The characters which have been used by Christophers (1933) hold good for Indian forms only. Using the keys given by Christophers (1933), Evans (1938) and De Meillon (1947), Reid (1968) made a new key which is reproduced below:

1.	With a single row of cibarial teeth not differentiated as rods and cones, or rarely without teeth (Ethiopian, Oriental and Australian)	*Neomyzomyia* series
	—With cibarial teeth in two rows, one of rods and one of cones	*Neomyzomyia* series 2
2.	Cones without roots	3
	—Cones with roots	4
3.	Pediment of cones with a single row of teeth on the crest. Larva with one of the long pleural hairs of the metathorax branched. Adult with propleural setae. (majority Ethiopian, also mediterranean and Oriental)	*Myzomyia* series
	—Pediment of cones with two rows of teeth on the crest. Larva with both long pleural hairs of the metathorax branched. Adult without propleural setae. (except *A. rufipes*) (mostly Oriental, a few Ethiopian)	*Neocellia* series
4.	Adult abdomen with projecting lateral tufts of scales. (Ethiopian)	*Cellia* series
	—Not so	5
5.	Fore tarsi with fairly broad pale bands, end of abdomen with at least a few scales (Oriental and Ethiopian)	*Pyretophorus (Pseudomyzomyia)* series
	—Fore tarsi dark or with only small pale bands, end of abdomen usually without scales. (chiefly Mediterranean, also East Africa and North Indian)	*Paramyzomyia* series

Though Christophers (1933) had used the name *Pseudomyzomyia* for a group, Reid, following Edwards (1932), Evans (1938) and De Meillon (1947), prefers to use *Pyretophorus*, which according to him is an older name.

The species in India belonging to the different series are:

Neomyzomyia	*A. balabacensis, A. elegans, A. kochi* and *A. tessellatus.*
Myzomyia	*A. culicifacies, A. fluviatilis, A. minimus, A. varuna, A. aconitus, A. jeyporiensis, A. jeyporiensis* var. *candidiensis, A. majidi.*
Pyretophorus (=Pseudomyzomyia)	*A. sundaicus, A. subpictus, A. vagus*

Paramyzomyia	*A. turkhudi, A. multicolor.*
Neocellia	*A. moghulensis, A. stephensi* and its var. *mysorensis, A. maculatus* and its var. *willmorei, A. theobaldi, A. karwari, A. jamesii, A. ramsayi, A. splendidus, A. annularis, A. philippinensis. A. pallidus* and *A. pulcherrimus.*

A simplified key for identification of the Indian anophelines is given in Appendix I.

Subfamily Toxorhynchitinae

Only one genus, *Toxorhynchites,* is known with three subgenera, only one of which, *Toxorhynchites,* occurs in the Eastern Hemisphere, extensively from Africa to Australia.

General characters distinguishing genus *Anopheles* from other common genera of culicidae

Adults: The palps of both sexes are of similar length, almost equal to that of the proboscis. The palps of the male have the two distal segments enlarged to give a club shaped appearance.

The posterior edge of the scutellum is only slightly curved (often called bar shaped) and continuous without any marked division into lobes, and the hairs thereon are in a continuous series.

The axes of head, thorax and abdomen are nearly in a straight line. The posture at rest is characteristic, the axis of the body forming an angle with the surface on which the mosquito rests. Therefore, the abdomen is directed away at an angle from the surface. The angle varies to some extent. In some species, like the members of the *hyrcanus* and *barbirostris* groups, the body stands out prominently to form an angle of over 60°. In a few, like *A. culicifacies, A. culiciformis, A. sintoni,* etc., the body is almost parallel to the surface, giving the adult a *Culex*– like appearance.

Of the three acini of the salivary glands of each side, the middle one is sac-shaped instead of being tubular as in culicines.

The wings are either spotted or unspotted. The spotted wing, which characterises many species of anophelines, is not exclusive to the genus; nor do all anophelines exhibit it. For exmaple, *Orthopodomyia anopheloides* and *Culex mimeticus* have spotted wings, while *Anopheles aitkenii* and relatives have completely dark wings.

The fore tarsi have a single claw in the male.

In the female there is a single spermathecum while in the common culicines there are three. The abdomen is not covered by imbricated scales as in Culicinae though there may be some scattered non-overlapping scales on the tergites, even these being rare on the sternites.

Larvae: The body rests parallel to the surface of the water being supported by various hairs along the thorax and abdomen. The head is capable of being rotated at 180° so that at the time of feeding the ventral surface is on the upper side. The so called palmate hairs occur in many species on metathorax and most segments of the abdomen. Two (one on each side) reversible organs, called Nuttal's organs, occur on the first segment

of the thorax; they are fleshy and protrusible not easily seen in killed specimens.

There is no siphon at the posterior end as in Culicinae, but there is a dorsal spiracular plate on which are located two spiracles (segment VIII).

Pupae: Paddle is oval in outline and is undivided or only slightly invaginated and the tip has a pair of setae one of which is ventral and sub-apical. Seta No. 9 (Belkin's system) on abdominal segments III to VII are spinelike and located at the postero-lateral corners usually with branches on VIII. The trumpet, with margin, often bearing at least one cleft.

Eggs: The eggs are boat-shaped with an upper'deck' with the micropyle at one end and a fringe all round and usually with lateral, floats with transverse striations called ridges. They are laid singly.

3

Geographical Distribution

ZOOGEOGRAPHICAL REGIONS

The world can be divided into several natural zoogeographic regions based not only on the present distribution of the land mass but also on geological histories. It is well known that the fauna and flora of these regions are generally characteristic of them and sometimes include exclusive taxonomic forms. Wallace (1876) supporting Darwin's theory of evolution studied the distribution of animals, particularly of mammals, and formulated his well known ideas of zoogeographic regions. His delineation of the region largely holds good even today, with a few modifications based on further knowledge.

The present distribution of the members of the family Culicidae, subfamily Anophelinae, remarkably well corresponds with Wallace's classification of the regions. Each region has its own characteristic anopheline fauna with some expectable overlap at the margins of the adjacent regions. The overlap is particularly seen in respect of the Oriental region which is not separated from its neighbours to the east and west by such insurmountable barriers as oceans or large deserts.

Six easily definable zoogeographical regions are accepted by mosquito entomologists.

1. Nearctic region : North American Continent extending upto South Mexico.
2. Neotropic region : South American Continent and part of North America south of Mexico.
3. Palearctic region : Whole of Europe, Northern Asia including Siberia, Mongolia, Northern China and Japanese archipelago. Asian countries to the west of Pakistan and parts of Africa north of the Sahara Desert.
4. Ethiopian region : Africa south of the Sahara.
5. Oriental region : Southern and Southeast Asia from Afghanistan (part south of the Hindukush mountains) in the west, Pakistan, eastwards through India to Weber's modification of Wallace's line, passing between Sulawesi (Celebes) and Timor to the west and the Moluccas and Ceram Islands to the east. The region includes part of Afghanistan, Southern Iran, Pakistan, India, part of Tibet, Bhutan, Bangladesh, Burma, Sri Lanka, Thailand, Vietnam, Laos, Kampuchea, Malaysia, the main part of Indonesia

including Kalimantan, southeastern China, Taiwan and the Philippines.

6. Australasian region : Parts of Indonesia east of Weber's line including West Irian, New Guinea, Australia, New Zealand, Tasmania and all islands of the Pacific south of Equator.

The continent of Antarctica is not included in any region.

Some of the characteristic features of the anopheline fauna of these regions may now be briefly described. The writings of Christophers (1933), Freeborn (1949), Lane (1949), De Meillon (1949), Bates *et al.* (1949), Puri (1949) and the distribution lists given by Russell *et al.* (1963) have been very useful in this respect. Further, the catalogues by Stone *et al.* (1959) revised by Knight and Stone (1977), and the checklist of Oriental species by Stone and Delfinado (1973) have added more species to the lists provided by these authors.

The distribution of species by zoogeographical regions as compiled from several sources, is shown in Table 1. Forms which occur in more than one region are included only in their native regions.

Table 1. Number of Anopheles species in different zoogeographical regions

Subgenera	*Anopheles*	*Cellia*	*Kerteszia*	*Nysso-rhynchus*	*Lophopodo-myia*	*Stetho-myia*	*Total*
Nearctic	19	—	—	—	—	—	19
Neotropic	28	—	9	24	6	5	72
Palearctic	19	7	—	—	—	—	26
Ethiopian	9	103	—	—	—	—	112
Oriental	67	49	—	—	—	—	116
Australasian	7	13	—	—	—	—	20
TOTAL:	149	172	9	24	6	5	365

Nearctic Region: All anopheline species of this region belong to the subgenus *Anopheles* of which only 19 occur. *A. quadrimaculatus* is the most predominant species.

Neotropic Region: The anopheline fauna is far richer in the number of species than in North America, as many as 72 species being recorded. The genus *Chagasia* with four species, except one species which occurs in Mexico City, as also the *Anopheles* subgenera *Nyssorhynchus* and *Stethomyia* are characteristic of this region. So also are the members of the *Aribalzzagaia* group. There are also members of the subgenera *Lophopodomyia, Kerteszia, Arribalzzagaia, Myzorhynchella, Anopheles* and *Coelodiazesis*. Some members of the Nearctic fauna like *A. barberi* are known to overlap into this region. The total absence of the subgenus *Cellia* is noteworthy. The temporary invasion of a member of *Cellia*, viz. *A. gambiae*, into north Brazil in the 1930's and later its complete eradication needs to be mentioned.

It should be noted that Stone *et al.* (1959) and Knight and Stone (1977) do not

recognize all these as subgenera. The subgenera which they recognize are only *Stethomyia, Anopheles, Nyssorhynchus, Kerteszia, Lophopodomyia* and *Cellia.* The subgenus *Myzorhynchella* is regarded as a synonym of *Nyssorhynchus, Arthuromyia* as a synonym of *Lophopodomyia,* and *Arribalzzagia* and *Coelodiazesis* as synonyms of *Anopheles.*

Palearctic region: The anopheline fauna of the northern parts of this large region consists only of a few members of the *maculipennis* complex of the subgenus *Anopheles* but the Mediterranean part has a richer fauna including, apart from several members of the *maculipennis* complex, many characteristic forms such as *A. hyrcanus, A. multicolor, A. superpictus, A. sergentii, A. pharoensis* and *A. coustani.* There are in all 26 species. Both subgenera *Anopheles* and *Cellia* occur. Though *A. gambiae,* one of the major species of the Ethiopian region, did invade lower Egypt once, it seems to have disappeared there. The fauna of this region is interesting to the students of the mosquitoes of India because in their distribution some of them overflow into their sub-region. A few species which are essentially of the Oriental region also occur in the West Asian countries, viz. *A. culicifacies, A. stephensi,* etc.

Ethiopian region: The Ethiopian region is known for the distinctiveness and homogeneity of its fauna and is generally regarded as very ancient (De Meillon, 1949). Owing to its comparatively well defined separation from the Palearctic region, the anopheline fauna is quite distinct and the species do not show much overflow into the latter. Only the subgenera *Anopheles* and *Cellia,* which are also found in the Indian sub-region, occur in this region. 112 species are listed and most of them are exclusive to this region, but some like *A. pharoensis* and *A.coustani* overflow into the southern Mediterranean sub-region of the Palearctic.

Oriental region: The Oriental region is rich in its anopheline fauna, which include both the subgenera *Anopheles* and *Cellia.* It should be noted that Christophers had used *Myzomyia* as a subgeneric name and, except for the group *Cellia,* all other groups of *Myzomyia,* viz; *Neomyzomyia, Myzomyia, Pseudomyzomyia, Paramyzomyia* and *Neocellia,* occur in this region. The group *Cellia* itself does not occur. These groups are all now merged in the subgenus *Cellia* Theobald, by Stone *et al.* (1959) and Knight and Stone (1977). When dealing in detail with Indian species, these group names will be used because of convenience. The members of each of the group have some very clear affinities among themselves. Puri (1949) had included 77 species in the fauna of this region, but the number has now increased to 116 because of several more recent investigations (Stone and Delfinado, 1973). According to them, there are several species which overlap into the Oriental region from the eastern part of the Palearctic region, such as *A. multicolor, A. dthali, A. turkhudi, A. sergentii, A. pulcherrimus* and *A. superpictus,* all essentially Mediterranean forms. None of the essentially Australasian fauna occurs in the Oriental region, but a few of the oriental species do overflow into the neighbouring areas of the Australasian region. The anopheline fauna of the Indian subregion is dealt with in detail later.

Australasian region: This region does not show such extreme isolation (as do the Neotropical and Ethiopian regions) in regard to its anopheline fauna as would be

expected from the known isolation of its mammalian fauna. The anophelines include members of the subgenera *Anopheles* and *Cellia* with the preponderance of the group *Neomyzomyia*. However, of the 20 species of *Anopheles* listed as occurring in this region, four, viz. *A. subpictus, A. pseudo barbirostris, A. tessellatus* and *A. karwari*, are from the Oriental region. *A. subpictus* and *A. karwari* are perhaps of recent introduction. However, there is some overlapping in the occurrence of certain species on either side of Weber's line. The genus *Bironella*, with eight species, is exclusively the inhabitants of this region; seven of them occur in New Guinea and one in the Solomon Islands. One of these species, *B. gracillis*, occurs in the northern part of Australia also.

Paleontology — Fossil forms: Several species of *Culicidae* have been found as fossils, particularly in the oligocene and eocene strata from places like England, (Isle of Wight), U.S.A. (Wyoming and Colorado), Germany, France, etc. Some of these mosquito fossils are embalmed in amber. Knight and Stone (1977) made a list of 16 species which can be definitely identified as members of *Culicidae* as we know the family today. They belong to the genera *Culex* (8), *Mansonia* (3), *Aedes* (1), *Toxorhynchites* (1), *Culicites* (2), and *Anopheles* (1). *Anopheles rottensis* Statz (1944) has been recorded from the upper oligocene in Germany. No fossil forms have been recorded from India. The material is hardly sufficient to give an indication of phylogenetic or distributional trends. The heavy odds against flying insects like mosquitoes becoming fossils have often been emphasized. There is no reason to doubt that mosquitoes have been in existence from times much earlier than the oligocene.

Because of the paucity of fossil forms, phylogenetic relationships of the present forms can be surmised only on the basis of the existing abundance and distribution of the species in different parts of the globe and of morphology. From all evidence, the subgenus *Anopheles* is regarded as the most ancient of all subgenera. This is based not only on the worldwide distribution and greater distinctiveness of forms, but also on hypopygial characters. Subgenus *Cellia* seems to be a later development. The fact that it is completely absent from the New World, further supports the idea. It also seems to be expanding in the numbers of the species and the areas of its distribution.

Invasion: As already described the anopheline fauna of the different regions of the globe are largely characteristic of the regions concerned though some overlapping occurs in their margins. In recent times there have been few examples of *Anopheles* species of one region transgressing effective natural barriers, such as oceans, deserts and high mountains, to invade other regions. A notable example was the appearance of *Anopheles gambiae* in Brazil in 1930. It invaded the south American continent probably accidentally by ship. In about seven years' time, it slowly invaded about 300 kilometres inland and caused an outbreak of malaria. The species was eradicated by the joint efforts of the Government of Brazil and the Rockefeller Foundation (Soper and Wilson, 1943).

In India, while there is no recorded evidence for such an invasion, there is the notable example of *A. sundaicus* which is strongly suspected to have become intro-

duced in recent times from the neighbouring maritime countries and later spread into the coastal areas of West Bengal, Orissa and Andhra Pradesh causing severe malaria outbreaks. It is usually difficult to pin-point the actual means of such invasions but adult mosquitoes as well as larvae are known to be carried by trains and boats. With the introduction of the modern air traffic, the chances of such intercontinental introductions have become enhanced. *A. sundaicus* had, however, been seen in the Andamans even in the earliest surveys.

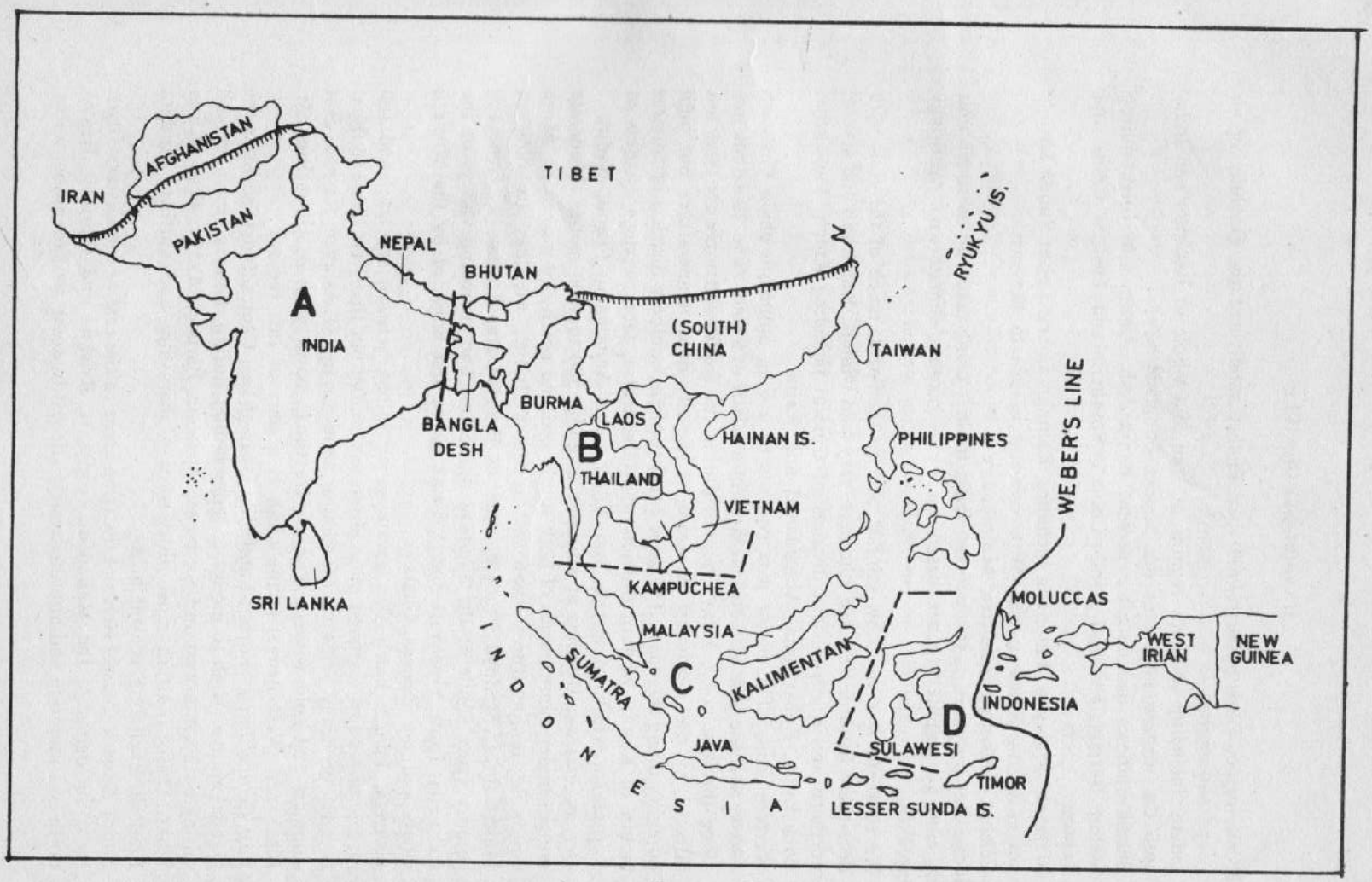
Transport of mosquitoes between international airports is now well documented (Highton and Van Someren, 1970). Several species of mosquitoes have been recovered in some countries carried on aircraft or vessels. Basio (1973) made a list of such instances. The references to anophelines are:

Species	*Country of recovery*
A. baezai	Guam.
A. lesteri	Guam.
A. sacharovi	Cyprus.
A. subpictus	Philippines, Guam.
A. tessellatus	Guam.
A. gambiae	Kenya, Brazil

There are several studies that have been reported on this subject. In one study made in the Manila International Airport in the Philippines between 1964 and 1968, a total of 534 adult mosquitoes, 138 adult flies and 45 moths were recovered from a total of 14,246 aircrafts inspected (Basio, *loc. cit.*). Most were dead specimens but a few live mosquitoes were also found. Three species, viz. *A. subpictus, Culex bitaeniorhynchus* and *C. fatigans*, were actually collected alive. All these have some vectorial potentialities.

A thorough and systematic study of the passive introduction of mosquitoes from other countries into the Indian airports and sea ports had become an immediate necessity. Current international sanitary regulations require that all international airports must be kept free of *Aedes aegypti* and *Anopheles* species which are vectors of diseases. Neglect of this provision is a violation of these regulations.

Oriental region: Sub-divisions (Map 1): The Oriental region as now conceived and illustrated by Stone and Delfinado (1973) is essentially similar to that given by Christophers (1933). However, it is necessary to include Afghanistan, south of the Hindukush mountains, in the Oriental region, because of the features of its anopheline fauna. While the fauna north of the Hindukush is that of the Palearctic, the fauna south of the Hindukush is typically Indo-Pakistani (Ramachandra Rao, 1951). In the east, Wallace's line used to pass between Kalimantan (Borneo) and the Islands of Bali in the west and Sulawesi (Celebes) and the Island of Lambok towards the east. Weber's line now passes between Sulawesi and Timor Islands to the west and the Moluccas and Ceram to the east. Consequently, the entire Sulawesi and the Islands of Sumba, Flores and Timor are now included in the Oriental region. Still the Indonesian West Irian and Papua form part of the Aus-



Map 1 Oriental region (a) Indo-Pakistan sub-region (b) Assam, Burma, China sub-region (c) Malaysian sub-region (d) Sulawesi sub-region.

The northern boundary of the Oriental region is demarcated by a thick line with cross hatching and the boundaries of the sub-regions by interrupted lines.

The territorial waters of India extend into the sea to a distance of twelve nautical miles measures from the appropriate base line.

tralasian region. Christophers (1933) had further subdivided the Oriental region into four sub-regions:

1. Indian: including southern regions of Iran and whole of Pakistan and India upto the western border of the present Bangladesh.
2. Burma-Chinese: including the present Bangladesh, Assam and neighbouring States, Burma, Thailand, "Indo-China", Southern and Eastern China and Taiwan.
3. Malayan: Malaysia, Indonesia including Kalimantan and Lesser Sunda Islands but excluding Sulawesi and Moluccas and islands to the east.
4. Celebes: including Sulawesi, Moluccas, etc.

India: For purposes of this volume, India itself is being taken up as single unit with nine geographical zones based on physiography, climate and hydrology (Map 2).

1. *North Western Zone*: Low rainfall, arid to semi-arid, much of the zone with desert-like feature. Very hot summers, very cold winters: Rajasthan and several northern and north-western districts of Gujarat including Kutch, Mehasana, Banaskanta, Subarkanta, Ahmedabad and Kaira.
2. *Western Zone*: The major part consists of a long mountain chain (Western Ghats) and the rest of a coastal strip of a width of about 10 to 50 kilometres along the west coast. Heavy rainfall during the south-west monsoon and not affected by the north-east monsoon. Generally mild temperatures but high humidity along the coast. The zone consists of the southern districts of Gujarat (south of Kaira), western districts of Maharashtra, Goa, western districts of Karnataka, whole of Kerala and Nilgiris and Annamalais (Tamil Nadu).
3. *Deccan Plateau*: Uplands at altitudes of about 500 to 1000 metres. Moderate rainfall under the influence of both south-west and north-east monsoons. More equitable temperatures than in northern India. Includes uplands of Maharashtra, Madhya Pradesh, Andhra Pradesh and Karnataka. Generally country lying south of the Vindhya mountains and including valleys of the Narmada, Tapti, Godavari and Krishna rivers and bounded by the Western Ghats and the Eastern Ghats.
4. *Gangetic Valley*: Fairly flat terrain at elevations between sea level and 500 metres with a few scattered hills. Moderate rainfall but the rivers liable to flood because of heavy rains or of melting snow in the Himalayan regions. Hot summers and cold winters. Punjab, Haryana, major parts of Uttar Pradesh, Bihar and West Bengal (Bangladesh is a part of this region).
5. *East Central India*: Forested and hilly areas of East Central India including the Dandakaranya. High to moderate temperatures during summer, heavy rainfall during both south-west and north-east monsoon: Parts of Madhya Pradesh and Bihar, Orissa, eastern most districts of Maharashtra and northern Andhra Pradesh, excluding coastal area.
6. *South Eastern Coastal Areas*: Low lying areas to the east of the Eastern Ghats including deltas of the Mahanadi, Godavari, Krishna and Cauvery. Heavy north-east monsoon and also moderately affected by south-west monsoon; warm

climate throughout the year, with very hot summers. Extending from Orissa through coastal Andhra Pradesh and entire Tamil Nadu except Nilgiris and Annamalais.

7. *Brahmaputra Valley*: Both lowlands along the Brahmaputra and also high lands of the neighbouring hill states; heavy south-west monsoon, warm and humid throughout the year, but cold winters in the highlands. Assam and neighbouring southern states of Meghalaya, Mizoram, Nagaland, Manipur and Tripura.
8. *Himalayan Zone*: Partly alpine and partly subalpine mountain regions of Himalayas: Generally cool weather in the summers and very cold snowy weather in winter. Extends from Kashmir through Himachal Pradesh, parts of Uttar Pradesh, and West Bengal, Sikkim, Bhutan, Arunachal Pradesh and Nagaland.
9. *Outlying Areas*: The two major island groups, viz. (1) Andaman and Nicobars (2) Lakshadweeps (Laccadives) are not parts of any of the above zones and are to be dealt with separately. Sri Lanka has all the features of the adjoining zone of India, viz. the Tamil Nadu coast.

Anopheles fauna of India and its relationship with those of neighbouring countries

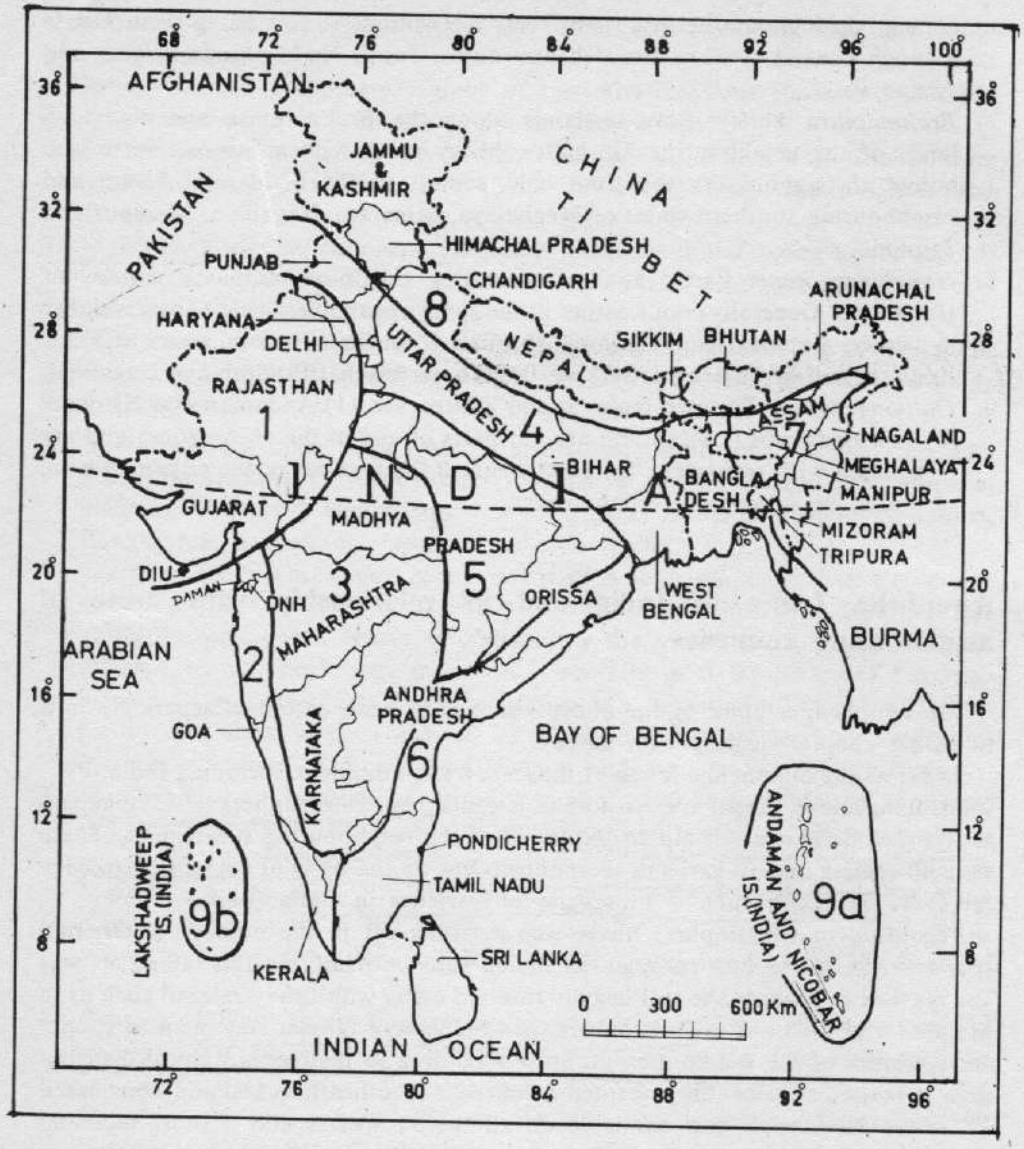
The anopheline fauna of the above zones have been described separately in a following chapter dealing with surveys.

So far as the anopheline fauna of the Indian sub-continent, including India, Pakistan, Bangladesh, Nepal and Sri Lanka is concerned, Christophers (1933) made an analysis of their relationship to the fauna of the neighbouring sub-regions. There were 42 species and 10 varieties then known but on the basis of current knowledge there are 51 species and 7 subspecies or varieties in India itself.

According to Christophers, there was a falling off in the number of Oriental species from east to west through the Indian sub-continent, but this falling off was less marked as regards the still heavily forested areas with heavy rainfall such as in Malabar and Sri Lanka. Christophers then prepared a tabular statement of species and varieties of the Indian area grouped according to their geographical distribution. A revised statement is presented below with modifications and additions based on newer knowledge now available on all the 51 species and 7 subspecies and varieties.

In preparing a list such as this, one meets with some difficulties. Except for a few species, the rest occur in more than one geographical area. India, being a part of the Oriental region, has many species which occur both to the west and to the east. However, a broad classification is presented here. The species are divided into three main categories:

I. *Indian elements:* i.e., species which have their main range of distribution in India.



Map 2 India and adjoining countries : Zones (1) North-western zone, (2) Western zone, (3) The Deccan plateau, (4) Gangetic valley, (5) East Central India, (6) South-eastern coastal areas, (7) Assam and adjacent states, (8) Himalayan zone, (9) Island zones: (a) Andaman and Nicobars, (b) Lakshadweep. Based upon Survey of India outline map printed in 1979 with permission of the Surveyor General of India The territorial waters of India extend into the sea to a distance of twelve nautical miles measured from the appropriate base line.

The boundary of Meghalaya shown on this map is as interpreted from the North-Eastern Areas (Reorganisation) Act. 1971, but has yet to be verified.

II. *Western elements:* i.e., species with their main range of distribution in countries to the west.

III. *Southeast Asian elements:* i.e., species with their main range of distribution in Southeast Asian countries.

I. Indian elements:

(a) Wide occurrence in India with extension of distribution both to the west and east.

A. annularis
A. culicifacies
A. maculatus var. *willmorei*
A. nigerrimus
A. splendidus
A. subpictus

(b) Wide distribution in India, but spread out mainly into countries to the west.

**A. fluviatilis*
**A. stephensi*
A. barianensis
A. moghulensis
A. stephensi var. *mysorensis*
A. turkhudi

*A slight but negligible extension into Burma.

(c) Wide distribution in India but extending eastwards only:

A. aitkenii
A. insulaeflorum
A. jamesii
A. karwari
A. tessellatus
A. vagus
A. jeyporiensis
A. pallidus

(d) Distribution in India largely in the eastern parts and extending further to the east.

A. bengalensis, A. jeyporiensis var. *candidiensis, A. ahomi*

(e) Predominantly or exclusively Indian species.

(i) *Wide distribution*

*A. interruptus, A. majidi, *A. varuna*

*with slight but negligible extension to Burma.

(ii) *Localised species*

A. elegans, A. culiciformis, A. sintoni, A. gigas, A. pinjaurensis

(iii) *Alpine species*

A. lindesayi, A. l. ssp. *nilgiricus, A. gigas* var. *baileyi, A. gigas* var. *simlensis*

II. Western elements found in India

A. dthali, A. pulcherrimus, A. multicolor

III. Southeast Asian elements occurring in India

A. aconitus
A. balabacensis
A. pediateniatus
A. sinensis

**A. barbirostris*	*A. philippinensis*
A. barbumbrosus	*A. minimus*
A. argyropus	*A. maculatus*
A. crawfordi	*A. ramsayi*
A. nitidus	*A. kochi*
A. umbrosus	*A. theobaldi*
A. sundaicus	*A. roperi*

*It extends very slightly into Pakistan to the west.

Total = 51 species
1 subspecies, and
5 varieties (*A. subpictus* var. *vadakadiensis* is excluded from the list).

Names of *A. superpictus*, *A. gigas refutans*, *A. hyrcanus*, *A. leucosphyrus*, *A. pseudobarbirostris*, *A. sergentii* and *A. habibi* are not included as they do not occur in India.

General considerations: The factors which influence and determine the occurrence of certain species of animals in the particular regions of the world have been the subject of much discussion by biologists ever since the times of Darwin and Wallace. Generally speaking, in the course of evolutionary history, many new forms arise either by natural variation and selection or mutation. Their survival and spread depend upon their ability to withstand the environment. Certainly the forms which had arisen and have been eliminated are many times more than those which have survived. Geographical isolation is one of the important factors that have favoured the evolution of new forms. The process is undoubtedly continuing even today but is not noticeable by us because of our limited range of experience. Modern evolutionary theory based on an intimate understanding of ecology and genetics can offer reasonably satisfactory explanations for many of the known features of distribution of species of animals and plants, as well as for their bionomics. (Also see Chapter 6).

4

Biology of Anophelines

The world literature on the biology of mosquitoes including that on anophelines is enormous. Reviews by authors such as Bates (1949), Horsfall (1972), Muirhead Thomson (1951, 1965) and others, to name a few have dealt exhaustively with several aspects of the biology on a global basis. The work of Reid (1968) and Harrison and Scanlon (1975) have referred to many features of the biology in Southeast Asian countries. In India itself, the small volume "Vectors of Malaria in India" by several authors, published by the then Indian Society for Malaria and other Mosquito-borne Diseases (1961) has fairly well dealt with biology of ten vector species as known till then.

No attempts will be made in this section to review the subject on a world-wide basis, but only to describe the behaviour and ecology exhibited by anophelines of India in a general manner. More detailed treatment of the subject will be found in the chapter dealing with individual species.

1. Prevalence

Anopheline mosquitoes are found in all parts of India from the sea level upto an altitude of 3,530 metres where *A. gigas* var. *simlensis* has been found at Kedarnath in U.P. Himalayas (Bhat, 1975b). Christophers (1933) has mentioned that Dr. Strickland had found a variety of *A. gigas* (probably *simlensis*) at an altitude of 11,000 feet (3,350 metres) near the Indo-Tibetan border, actually attempting to bite (man?). *A. maculatus* var. *willmorei* had been collected at heights of 2,740 metres in many places in the Himalayas. Another species of high altitudes is *A. gigas* var. *baileyi* found at 2,740 metres in the Sikkim area.

There are records of mosquitoes found in deep mines particularly culicine mosquitoes. They have been found at depths of over 1000 metres in the Kolar Gold Mines in Karnataka. However, anophelines have been found only at depths in the coal mines of Bihar. Azeez (1965) has recorded the following species:

A. annularis and *A. vagus* at depths of 300 to 600 feet (92 to 184 metres)

A. culicifacies
A. nigerrimus
A. stephensi
A. subpictus

at depths of 300 feet (92 metres)

Culex fatigans was found breeding at 600 feet (184 metres) below ground level.

Generally speaking, the number of species at the sea coast or in the plains is

smaller than in the hills and foothills. For example, while only 12 species are found in Thanjavur District in Tamil Nadu, as many as 32 species are found in the Nilgiris in the same State. What the coastal areas or the plains lack in the variety or species is, however, made up by the abundance of the common species. Most of the rare species occur in the hills, as for example *A. gigas* and its varieties and *A. culiciformis, A. sintoni, A. elegans*, etc.

There is a gradual falling off in the number of species from east of India, bordering south-east Asian countries, to the west in areas bordering Pakistan.

There is not much evidence as to the height above the ground level at which anophelines can be found in India because very few comparative studies have been made of anophelines at ground levels and in tree canopies in the forest or in upper floors of multi-storeyed buildings. However, it is known that *A. stephensi* can breed in overhead water cisterns in tall buildings six or seven storeys high in Bombay city.

Except in the extreme north under Himalayan conditions, no part of the year is totally inimical for the life of anophelines. In peninsular India and eastern India, where temperatures are moderate, most common species of *Anopheles* occur throughout the year. But the abundance of any species depends more upon the availability of breeding places than season. The monsoon and the immediate post-monsoon months provide the best opportunity for many species of *Anopheles* to live and multiply. In certain other areas, such as irrigated areas, the season of irrigation, which may or may not coincide with the monsoon months, provides optimum conditions for high anopheline densities. In certain riverine areas, particularly in the Deccan where there are sharp differences between the monsoon months and pre-monsoon dry months, the pools in beds of rivers and streams provide favourable breeding places for certain species like *A. culicifacies*. The season of greatest abundance of some species is in the months of April and May, in spite of severe atmospheric conditions.

Species, like *A. annularis*, which need still waters with good growths of vegetation generally are more prevalent in the non-monsoon months, when there is a greater concentration of water with richer plankton and growths of higher vegetation.

Most species of mosquitoes can tide over really unfavourable seasons by either finding ecological niches with microclimates which are suitable for their survival or in the case of larvae by breeding in habitats which are not normal to them. There is no evidence that the eggs of any species of *Anopheles* can survive prolonged desiccation as do those of *Aedes aegypti*. The phenomenon of hibernation and aestivation will be discussed later. But Christophers (1933) has mentioned an observation by Kenrick that larvae of *A. jeyporiensis* (more probably *A. moghulensis*) burying themselves in wet sand and emerging again after rains.

Physiography and climate play an important role in the seasonal prevalences of most species. This is best illustrated by the conditions in North Kanara District of Karnataka where *A. fluviatilis* is the vector. (1) There is a long coastal belt which is warm and humid but with very few breeding places suitable for the species and what little malaria occurs in that area is generally imported. (2) Parallel to it is a region of hills of the Western Ghats rising with a steep escarpment and character-

ized by mountainous terrain with deep valleys in some of which extensive arecanut cultivation is undertaken. This region abounds in innumerable perennial streams. In some cases, water actually oozes out from the earthen banks of such streams. These perennial streams and channels provide ideal breeding place for the species, but during the monsoon months of June to September heavy rains, sometimes exceeding 500 cms a year, result in the flushing away of the few *A. fluviatilis* larvae which may succeed in persisting. At this season, the species takes shelter in small numbers in shallow wells and even in tanks which are normally not the preferred habitats. Soon after the monsoon, breeding of the species intensifies and continues to increase till the onset of the next monsoon in June.Therefore, the season of the greatest abundance of the species is between October and June.(3) Still eastwards is the gently undulating country which tapers off into the Deccan plateau with an average rainfall of less than 100 cms. This region is characterized by extensive rice cultivation during the monsoon season between June and October. *A. fluviatilis* breeding occurs during the monsoon months not only in the channels but also in the rain-fed rice fields. The rainfall is not enough to flush away the larvae. Thus in this region the season of highest prevalence of *A. fluviatilis* is the monsoon months. Soon after the monsoon, the entire area dries up and there are very few perennial streams and channels. These variations in the breeding of *A. fluviatilis* have been described to illustrate how abundance of the same species may be so different even within the confines of one district. The seasons of prevalence of *A. minimus* are very similar to those of *A. fluviatilis.*

An interesting observation has been made in respect of *A. philippinensis* by Iyengar in Bengal. It breeds preferentially in swamps, tanks and jheels covered with vegetation. But the species is most abundant in the seasons when sub-soil water level is low. When the sub-soil water rises due to irrigation or rainfall, the abundance of the species declines. The exact mechanism of this habit is not known, but it is another illustration of how climate and terrain can influence seasonal abundance of anophelines.

In the forested areas, where many species breed either in tree-holes, such as *A. culiciformis*, or on the water-soaked ground pools in deep shade, such as *A. balabacensis*, the monsoon and immediate post-monsoon seasons are the most favourable. How they manage to survive the dry season is somewhat a mystery, though it may be noted that in the forested areas, there is some rain in most months of the year or there is very heavy dew which keeps the sub-stratum in the holes moist.

Species, like *A. stephensi*, which breed in wells have suitable breeding places available throughout the year and generally wide seasonal fluctuations are not noticed in them. Even so,some seasonal changes have been noticed in respect of this species.

In the north-western parts of the country, such as Gujarat and Rajasthan, semi-arid conditions prevail. In normal years, *A. culicifacies* is found in small or moderate numbers in nullahs, wells and tanks. But in years of unusually heavy rainfall, vast sheets of water are formed on the sandy soil leading to exceptionally high densities which result in widespread regional malaria epidemics.

In this connection reference may be made to the observations of Covell and Baily (1930) in Sind where wide fluctuations in the numbers of *A. culicifacies* occur from year to year depending upon rainfall. Regional epidemics of malaria in the Punjab [Gill (1928), Yacob and Swaroop (1941)] had also been attributed to the occurrence of unusually high densities of this species following heavy rains and high humidity.

Why certain species are more abundant in some regions and are absent or less abundant in others is a question which needs an understanding of evolutionary processes — a subject not particularly well studied with respect to anophelines.

Great changes in abundance are sometimes noticed as a result of natural trends extending over years. The case of *A. sundaicus* in India is an example of such a change. It has disappeared from the entire Orissa and Andhra Pradesh coast where it was prevalent till the early 1950's. The example of *A. minimus* will be dealt with later (Chapter 10).

More detailed information on the prevalence of anopheline species, both seasonal and regional, is given under each species.

2. Daily Life of an Adult Anopheles

The daily life of an *Anopheles* adult consists of several phases: viz.

(a)	Day-time resting in shelters:	from a little after dawn and to a little before dusk.
(b)	Crepuscular activity including exit from shelters followed by swarming and mating:	from a little before dusk and to a little after dusk, some activity at dawn.
(c)	Oviposition:	throughout night, but more frequently in the earlier part.
(d)	Feeding:	throughout night, but with variable peaks of activity, some species feeding in the earlier part, others in the middle or later part.
(e)	Flight and dispersal:	throughout the night from a little before dusk to a little after dawn.
(f)	Shelter seeking:	partly as a result of movements at night and partly at dawn and some movement within the shelter during day time.

The activities are performed in a rhythmic manner controlled both by endogenous rhythms and by responses to the daily 24-hour changes in the conditions of light and darkness. It is known that in mosquitoes the endogenous rhythm persists for a short time even after the periodicity of light and darkness is altered artificially, but soon the mosquitoes become adjusted to the new pattern of periodicity. The subject is a vast one and will not be discussed here.

In addition to the above activities which manifest themselves in locomotor movements of mosquitoes, there is the gonotrophic cycle which is also rhythmic in char-

acter. Though it does not manifest itself in locomotor activity, it plays a significant role in influencing the movements.The rhythm of gonotrophic cycle follows usually a 48 hour or 72 hour periodicity but with some variations due to season. This rhythm is also adjusted to the daily changes in light and darkness.

The daily activities of mosquitoes are also nowadays referred to as "diel" activity.

(a) Day-time resting habits

Most of the anophelines of India are nocturnal in their activities and spend the day-time in suitable shelters. Except for a few species which live in the forest and bite suitable hosts in the forests, like *A. umbrosus*, the rest of the species which are in close contact with man and his domestic animals frequently are found resting either inside houses, in mixed dwellings or in animal dwellings. Because many of these species can be found in man-made structures, an impression is liable to be formed that they are the only resting places. However, for a long time it has been known that several species of *Anopheles*, including some of the major vectors, are also found resting in outdoor shelters such as under culverts, along shaded banks of streams, in rock crevices, in tree buttresses, grass, bushes, etc. The degree of outdoor resting varies considerably between species and species and in the same species in different areas or seasons.

Among the major vectors of malaria, *A. fluviatilis* and *A. minimus* rest outdoors to a considerable degree. Being hill or foothill species, they have plenty of opportunities to find suitable shelters outdoors. The outdoor resting habits of *A. fluviatilis* have been observed both by the Bombay Malaria workers in North Kanara District and by Senior White group of workers in east central India. Viswanathan, Ramachandra Rao and Rama Rao (1944) estimated that about 60 per cent rested outdoors during summer and 40 per cent in cooler months. Such estimates, however, are applicable only to the area in which the studies were made. In Hassan District of Karnataka, Brooke Worth (1953) and Bhombore *et al.* (1956) however found little evidence of outdoor resting, differing from the North Kanara and east central India experience. They also found more adults of *A. fluviatilis* in cattle sheds than in houses, again differing from the observation of other workers. They suspected that the adults they were working with belonged to a different geographical strain. However, the work of Jaswant Singh and Mohan (1951) in the Nilgiris showed how much of movement towards outdoors could take place during night time itself.

A. culicifacies has been generally regarded as an indoor resting species because of the ease with which it can be collected in large numbers in houses and cattle sheds. But even very early observations made by Perry (1914) showed that many adults rested in bushes. Direct observations of outdoor resting have been made with this species by the present author in several places in Maharashtra, including Dhadgaon area of Dhule District where large numbers of *A. culicifacies* breed, rest and feed entirely out doors in the forest, leading to a persistent malaria transmission, because there are no man-made structures in which to spray DDT. In the forested

areas of Panchmahals District in Gujarat, Shalaby (1970) found that large numbers of *A. culicifacies* and *A. fluviatilis* resting in artificial pit shelters dug by him, strongly indicating a considerable degree of outdoor resting. The specimens collected were in all stages of gonotrophic cycle indicating that the outdoor resting was not a casual feature or only in respect of freshly hatched or unfed individuals. Even in the non-forested plains of the country, at Pattukottai, the present author had collected numerous *A. culicifacies* adults in earth-lined box-traps placed close to rice fields.

In respect of *A. balabacensis* it should be noted that it not only bites out of doors, but also predominantly rests outdoors. The adults gathered in biting collections either outdoors or indoors at night far out-number the adults found resting in human or animal dwellings (Sen *et al.*, 1973). Studies on *A. balabacensis* and *A. minimus* in Thailand (Ismail *et al.* 1974) have yielded much information. Using verandah trap huts, they found that *A. balabacensis* does not remain indoors to complete the gonotrophic cycle. About 95 per cent leave the huts after taking the blood meal. However, *A. balabacensis* has a greater tendency to bite man indoors than *A. minimus* which is reluctant to enter houses, but both species are outdoor resters. Previously it had been regarded that *A. minimus* was both endophilic and endophagic. Similar were the conclusions of Covell (1944) but Chang (quoted by Covell) had reported an exophilic habit. It would seem that either two races are involved or there are differences in different areas. Else, may be critical methods of study such as those applied by Ismail had not been used by earlier workers.

A. philippinensis is now known to be predominantly an outdoor rester (Rajagopal, 1976). Practically all other vector species, namely *A. annularis* and *A. varuna*, show evidence of some outdoor resting. With *A. stephensi*, very little outdoor resting is possible in highly urbanised areas, but adults of *A. stephensi* var. *mysorensis* normally behave like other species.

Among non-vectors, for example, *A. nigerrimus* is found rarely inside houses and cattle sheds even when abundant breeding is taking place nearby in places such as rice fields. Many are known to bite outdoors in the evenings. Even if they enter houses they leave before dawn. In a magoon type of trap which permits entry but prevents egress, Russell and Ramachandra Rao (1940) showed that large numbers of *A. nigerrimus* could be trapped, while the number collected in houses and cattle sheds was much smaller indicating a definite tendency of exodus to outdoor shelters. 5,399 *A. nigerrimus* adults were collected in the trap in one year while 123 were collected in houses and cattle sheds during the same period. Reuben (1971) has confirmed this in North Arcot District of Tamil Nadu; she collected 10,970 adults in a stable-trap in a five year period as against only 19 in cattle sheds during 1,922 man hours.

Therefore, generally speaking all anopheline populations have at a given time both indoor resting and outdoor resting components. Their proportions, however, vary considerably, not only depending upon the habits of the species itself but also on the environment, whether it is sylvan, rural or urban.

The terms *exophilic* and *endophilic* have been widely used to describe species

which have a tendency to rest outdoors or indoors respectively. Such a distinction seems to be an artificial one because the same species under different conditions can be highly endophilic or exophilic. The environment plays an important part in this regard.

Generally, it used to be surmised that the place of daytime shelter corresponded with the place of feeding at night. The adults were presumed to have remained in the shelters where they had fed. This is only partially true. Some females after feeding stay on in the shelter in which they take the blood meal, but a proportion go out before dawn. In some cases, as with *A. philippinensis* in Burnihat, Assam, all leave houses after feeding (Rajagopal, 1976).

It has also long been known that in the north western region where people mostly sleep outdoors during night in summer because of excessive heat, and where the cattle are also tethered outside, many mosquitoes, particularly *A. culicifacies* are found resting in large numbers inside houses and cattlesheds during daytime. This was further exemplified by observations in Afghanistan (Ramachandra Rao, 1951) where a similar outdoor sleeping habit of the people was universal during summers, but enormous numbers of *A. culicifacies* were found during daytime in houses and stables. The specimens included many which were freshly fed. In Ceylon Ariaratnam (1955) has found that when cattle are tethered outside human dwellings, there being no cattlesheds, many *A. culicifacies* adults which had fed on cattle were found resting inside houses during daytime. The precipitin tests on stomach bloods of *A. culicifacies* adults in Pattukkottai area, South India, collected from adults resting in pure human dwellings indicated that the percentage which had fed on man was only 2.5, the rest having fed on cattle. Obviously, many females which had fed on cattle out of doors had entered the human dwellings for shelter. Therefore while with some species the place of resting may also coincide with the place of feeding, it is not the general rule and it would not be correct to draw conclusions regarding the relationship between outdoor and indoor resting from numbers collected inside houses, etc. Environment as well as the innate habits of the species play a very important role in the selection of palce of resting (See under "Shelter seeking").

In Pakistan, Reisen *et al.* (1976a and b) have made some interesting observations on the diel periodicity and resting habits of mosquitoes in a village near Lahore, Punjab. Regarding daytime resting habits they found that *A. culicifacies*, *A. stephensi* and *A. subpictus* were almost completely endophilic in their resting habits and were rarely taken outdoors. *A. annularis* appeared to rest both indoors and outdoors although the degree of exophilism may vary seasonally. *A. pulcherrimus* seemed to have similar exophilic habits though the degree of exophily varied perhaps geographically. It may be noted that Onori *et al.* (1975) have found this species to be highly endophilic both in its resting and biting habits in Afghanistan.

Even during different times of the day Reisen *et al.* (*loc. cit.*) found variations in density of resting mosquitoes. For example, with *A. culicifacies* they found that the numbers of females gradually increased from about 32 for 15 minutes at 06.00 hours to nearly 57 till 12.00 hours and then a gradual fall to about 9 at 20.00 hours. With *A. subpictus* the highest density was at 09.00 hours (19.5 for 15 minutes) and

subsequently there was a gradual fall to 0.9 at 20.00 hours. Then there was a general fall till 18.00 hours followed by the evening exodus. The actual figures were:

Number of females collected for 15 minutes.

	09.00	12.00	15.00	18.00–19.15	20.00–21.15
A. culicifacies	32.0	57.5	48.5	32.3	9.0
A. subpictus	19.5	8.5	11.0	8.5	0.9
A. stephensi	13.0	1.5	8.5	2.0	0.6

Certain observations made on the relative numbers of *A. culicifacies* found during mornings and afternoons by Vishwanathan *et al.* (1951) and by Wattal (1962) will be discussed under that species. Wattal found an increase in numbers resting at lower levels in houses in the afternoons because it was cooler there than near the roof.

All sylvan species are predominantly outdoor resters, particularly the members of the *umbrosus, hyrcanus, barbirostris* and *aitkenii* groups which are not taken in any numbers in man-made structures. Even among the members of the subgenus *Cellia* in Malaysia, species such as *maculatus, kochi, aconitus, balabacensis* and *philippinensis* are outdoor resters. In South India, for instance, *A. tessellatus*, an uncommon species in houses and cattlesheds, is found to rest in good numbers on the walls of deep wells. The famous example of *A. minimus* var. *flavirostris* which is the main vector in the Philippines resting almost exclusively in outdoor shelters has been well documented (Russell, 1931).

Both males and females are generally found resting indoors as well as outdoors. The number of males is generally lower than that of females, mainly because of their short life span and limited dispersal. The presence of large number of males generally indicates the proximity of breeding places.

In Pune District, over a period of two years several species of anophelines were found resting outdoors by Ramachandra Rao and Rajagopalan (1957). The types of outdoor places searched were: rice fields, sugarcane fields, rock crevices, etc. Many species of mosquitoes, both culicines and anophelines were found and several species of anophelines were found resting outdoors.

The most direct method of determining outdoor resting habits is the collection of adults actually resting outdoors. Many such records are available in literature including Perry's (1914) observation that *A. culicifacies* adults were collected from under the shade of bushes. Vishwanathan *et al.* (1949) recorded 19 adult *A. fluviatilis*, which had already taken a blood meal in outdoor shelters while during the same time only 4 adults were collected in houses. Senior White (1957) also collected several adults outdoors. Bhombore *et al.* (1954) failed to collect a single specimen of *A. fluviatilis* outdoors in the Western Ghats of Karnataka State. Ismail *et al.* (1974) also collected adults of *A. balabacensis* outdoors and a few indoors. Sen *et al.* (1973) found practically no *A. balabacensis* in houses. All of them were found to leave houses after feeding. These differences could be due to existence of

geographic races or may be due to methods of collections. Adult mosquitoes tend to become concentrated inside houses which they enter to feed and it would be easy to collect them there, but in outdoor shelters, particularly in forests, they become very widely scattered and it would be very difficult to collect even a few specimens. It would be erroneous to compare directly the numbers collected in human habitations and cattlesheds with those collected outdoors.

It is not easy to make comparative observations on outdoor and indoor shelters in the same area over a long enough period. Therefore, the observations made by Ramachandra Rao and Rajagopalan (1957) are of special interest. For a two year period from April 1953 to March 1955, they studied the mosquitoes found in Pune District from four different localities, including Pune city. They collected and examined 49,851 mosquitoes of 59 species both anophelines and culicines. The data for anophelines they collected are summarized in Table 2.

Table 2. Anophelines collected in Pune District

	Indoors	*Outdoors*	*Biting man Outdoors*
	1	2	3
A. aitkenii		3	—
A. annularis	7	—	—
A. barbirostris	1	1	1
A. culicifacies	791	120	3
A. culiciformis	—	2	2
A. fluviatilis	199	107	4
A. nigerrimus	4	26	8
A. jamesii	48	13	4
A. jeyporiensis	14	2	—
A. karwari	—	—	1
A. maculatus	3	4	9
A. moghulensis	118	1	16
A. pallidus	13	1	—
A. splendidus	4	2	—
A. stephensi	11	—	—
A. subpictus	1106	68	3
A. tessellatus	80	39	2
A. theobaldi	11	5	1
A. turkhudi	52	14	—
A. vagus	22	12	1

One other species previously known, viz. *A. varuna*, was not collected during the study. While most species were found both indoors and outdoors, *A. subpictus* and *A. moghulensis* showed greater preference for indoor resting. No species was predominantly an outdoor rester but the high proportions of *A. culicifacies* and *A. fluviatilis* resting outdoors may be noted. *A. nigerrimus*, as expected, was collected more in outdoor shelter than indoors though their numbers were small.

To sum up, anophelines exhibit a wide range of variations in the indoor and

outdoor resting habits. Most species are found both indoors and outdoors. But their proportions may vary from place to place and season to season. There are both innate and external factors which influence the choice of the place of shelter.

(b) Crepuscular activity, swarming and mating

The crepuscular activity of anophelines is seen at dusk and dawn. It commences when they are released from the depressant effect of strong daylight. The dusk activity generally starts in the late afternoons, sometimes even a couple of hours before sunset. Mosquitoes become gradually sensitive and begin to move their legs, palps, antennae and wings. They may even start taking short hops within the shelter itself moving from place to place. The first real flight activity is the movement out of doors or out of natural shelters, into the open attracted by the weak light outside. This occurs a little before actual sunset. The next phase of activity, when there is still visible light in the open, is swarming and mating and in some cases of actual feeding outdoors. The next phase of activity is the biting activity which commences soon after the swarming ceases, but goes on late into the night or throughout the night. Almost similar flight activities, but in quite the reverse order, occur at dawn in a much weaker way. The dawn activity is followed by seeking shelters for day-time resting. The orientation mechanism which regulates this movement is one of positive phototaxy* to weak light.

It had long been known in other countries that mosquitoes of many species formed swarms during dusk and that mating took place during the swarming flight. The swarms are mainly composed of males and the females enter the swarms and mate with the males in flight and the mating couples drop down to the ground. However, till Ramachandra Rao and Russell (1938) actually observed and described the swarming and mating of *A. annularis* in South India, no observations had been recorded on Indian anophelines.

The swarms are usually found over some vertical object,a bush, a tree, a pole, a hut, an animal or over the heads of man. The males begin to gather as sun sets and during the twilight, however short it may be *A. annularis* swarms start when the light in the open comes down to about 2 foot candles. Numbers gradually increase and swarms consisting of as many as 500 or more have been seen.

With *A. annularis*, swarming started about 10 minutes before it became dark and continued for about 10 minutes more. When there was complete darkness, swarming activity ceased or at least could not be observed any longer.

Swarming of *A. subpictus* and *A. sundaicus* has been studied by Venkat Rao *et. al.* (1942) in Orissa. Later Venkat Rao observed swarming of *A. annularis* also. In the case of the latter he found an unbroken line of swarms for nearly 0.8 kms. The characteristics of the swarms were all similar but while swarms of *A. sundaicus* and *A. subpictus* were found at quite a distance from the Chilka Lake where they were breeding, swarms of *A. annularis* were much nearer the lake.

Swarming and mating of *A. culicifacies* were first observed and recorded by Russell and Ramachandra Rao (1942) in the large outdoor cage which they had built to

*The word "Phototropism" which is often used by entomologist is not appropriate. Tropisms are really "bending movements", Taxes are directed movements towards or away from stimuli. Phototaxy is the correct word to use.

study several aspects of the biology of the species. Swarms could be seen against the diminishing light of the western sky and mating pairs were examined. It was found that females which had taken a previous blood meal as well as females which had empty stomachs were actually mating.

Some very critical studies on this aspect of anopheles behaviour have been made in Pakistan, Quraishi (1965) studied in nature the swarming and mating of *A. stephensi* var. *mysorensis*. He found a high rate of copulation—about 400 copulations in a swarm of about 500-600 males each evening. Such intense mating has not been found with *A. annularis* or *A. culicifacies*, Reisen and Aslamkhan (1976) studying *A. culicifacies*, noticed that swarming started 20 minutes before sunset and ended 20 minutes after sunset, taking a much longer period than observed for the same species in South India. They noticed that 72 per cent of the females which entered the swarms had taken a partial blood meal either the same evening or on the previous night, but all females were nulliparous.

The mating behaviour of Indian anophelines in laboratory colony cages has been studied. Russell and Mohan (1939) have recorded that vigorous swarms occurred in cages each night approximately at 18.30 hours in a colony of *A. stephensi* maintained in Madras. They found in that species that (1) pairing was not necessarily dependent on ovarian development, (2) a blood meal was not essential for successful mating, (3) pairing may take place with nulliparous females and (4) the males attempted to mate with females of other species, e.g., *A. annularis*. Sometimes males of *A. stephensi* attempted to mate with males of their own species!

Certain observations have also been made on the mating in laboratory cages of *A. culicifacies* by Ansari *et al.* (1977).

It was for some time the practice to classify mosquitoes either as *eurygamous* or *stenogamous*. Eurygamous mosquitoes were those which needed large open spaces for swarming and stenogamous mosquitoes those which could mate in small confined spaces, a classification, which recent experience shows is not altogether perfect.

Light, sound, wind, humidity and rain all influence the degree of swarming activity. They will be discussed later under the respective species.

Swarming and mating in many species of *Anopheles* in Europe and North America have been observed and recorded, for example with *A. atroparvus*, *A. claviger*, *A. maculipennis*, *A. superpictus*, *A. multicolor*, *A. quadrimaculatus* and several others.

The exact orientation mechanisms which stimulate swarming activity of males are not fully understood. In the present author's opinion, it may be a kind of an orthokinetic response to sudden bright light after coming out of daytime shelters, which in the late evenings are darker than under the open sky. The first reaction of *Anopheles* in the evenings seems to be an exit from the shelter attracted by the weak light of the sky (positive phototaxy to weak light). Soon after coming into the open, the sudden change to bright light may stimulate the flight activities. In certain unpublished observations which the author made on *A. maculipennis* and *Culex molestus*, he had found that when mosquitoes which had been kept in lamp chim-

neys in total darkness were released into a bell jar (the bottom half of which was shaded by black paper) at any time of the day, from 10.00 hours to 15.00 hours, a few brief seconds of quietness ensued. It was followed by a vigorous flight activity of upto 10 minutes, at the end of which all mosquitoes went down into the shaded portion of the bell jar. However, when the adults which had been kept in open light in uncovered glass chimneys were released there was no flight activity at all, and the mosquitoes simply rested on sides on the jar without any special relation to the shaded portion or the unshaded portion. When mosquitoes were kept continuously in the bell jars, they were found resting on the shaded portion during daytime and after 16.00 hours they came out one by one into the unshaded upper part. About 18.30 hours there was a flight activity simulating swarming. At dawn, the mosquitoes gradually went down into the shaded portion to rest. This almost corresponded with the type of behaviour noticed in natural shelters. The flight activity at dusk and dawn was not so vigorous as when sudden changes in light occurred. These studies seemed to indicate that if the change was sudden from complete darkness to bright light it resulted in the flight activities stimulating swarming. The author has been unable to carry out any experiments with Indian anophelines so far, a study which would yield very useful information.

Swarming seems to be a mechanism evolved in mosquitoes, indeed by several other speices of insects including May flies (Ephemeroptera), for facilitating the coming together of the two sexes. It is a purposeful non-directional flight activity (orthokinesis).

Swarming activity of mosquitoes in nature in the early part of the dawn, particularly with *Culex fatigans*, has been observed, but observations on anophelines are absent.

(c) Oviposition

In the laboratory most anopheline females can be induced to lay eggs on wet filter paper when confined in small tubes or vials or on water surfaces in small bowls or basins when held in cages. Generally pans or basins with a black background are preferred to white ones. Sometimes floating cork rings can provide a foothold for the gravid females. The establishment of colonies of *A. stephensi, A. tesselatus,* etc., in India and of species like *A. atroparvus, A. quadrimaculatus,* etc. in other countries have been possible because of this ease of egg laying.

However, not all anopheline species are so easily colonized. Besides other reasons such as failure to mate in small cages, the need to have a larger space for egg laying, has been found to be a factor which has hindered laboratory colonization of many of them.

The egg laying habits in nature have not been adequately observed with any but a couple of species. The actual egg laying act in nature has not been described except for *A. culicifacies* though such observations have been made with some culicine and aedeine species. According to Bates (1940) many anopheline females will alight on the water surface and drop eggs one by one, when confined in cages. Bates (1949) quoted J.S. Kennedy to have observed a kind of dance in caged mosquitoes (presumably *A. atroparvus*) when working in Albania.

Russell and Ramachandra Rao (1942c) made the first direct observations on the ovipositing dance performed by *A. culicifacies* gravid females in natural conditions. The female performed an up and down "hovering dance" a few inches above the water surface of borrow pits while dropping the eggs. The eggs were actually collected in petri dishes floated under the dancing female.

However, there is no doubt that the vast majority of anophelines of India lay eggs when resting on the bank or on a blade of grass or on vegetation or when resting on water surface itself. The eggs are laid singly and not in rafts as in the case of certain culicines. The eggs disperse themselves over the surface of water and due to hygroscopic attraction come together either in a long ribbon-like arrangement, the eggs touching each other on the sides, or form patterns, triangles, squares or pentagons, the ends touching each other.

Under laboratory conditions the gravid females can sometimes be induced to lay eggs if they are thrown with some force on the surface of the water from a vial or test tube, or if tobacco smoke is blown into the vial in which they are held.

In some species there is evidence that the females indiscriminately deposit eggs in many types of breeding places and the eggs hatch but the larvae grow only in suitable types of water. But there is also evidence that a degree of selection is made by the females.

Among the earliest studies on Indian anophelines is one of Mehta (1934b) who worked with *A. culicifacies* and *A. subpictus*. *A culicifacies*, which in nature breeds in comparatively clear water, when given an opportunity to select waters with different amounts of free ammonia and ammonium carbonate, laid eggs indiscriminately, even in water containing 6.6 ppm. of saline ammonia. On the other hand, *A. subpictus* which can often breed in highly contaminated waters in nature, preferred to lay eggs in water with a high ammonia content. However, both these species are found in a wide variety of breeding places in nature and their eggs are perhaps scattered widely and the larvae develop in suitable habitats.

The absence or scarcity of *A. culicifacies* in the rice fields with plants over 30 cm in height could be due to mechanical obstruction offered by the plants for the performance of the "ovipositing" dance. Eggs artificially introduced into the rice fields however grew normally (Russell and Ramachandra Rao, 1942a). At the same time the presence of rich plankton and organic amorphous matter in the water of borrow pits deflected *A. culicifacies* from egg laying; so did the presence of blue green algae (Russell and Ramachandra Rao, 1942d). Therefore, *A. culicifacies* may also show some preference or avoidance in egg laying.

The experiments of Pal (1945b) showed that in the laboratory, when provided with waters of different temperatures *A. culicifacies* did show some choice when extreme temperatures were offered but not when the differences were slight.

In a study in Pakistan by Reisen and Siddiqui (1978), *A. stephensi* females laid more eggs on water in which larvae had been reared previously than on distilled or well water possibly responding to either increased ammonia concentrations or odours elicited by bacteria. Such a preference had been noticed for other mosquitoes by earlier workers. The one in India of interest to Indian workers would be the

observations by Soman and Reuban (1970) on the preference of *Aedes aegypti* to lay eggs in water in which the larvae were being reared. According to Reisen and Siddiqui (*loc. cit.*), the gravid *A. stephensi* did not distinguish between rearing trays with crowded larvae or uncrowded larvae. However, rearing water was not significantly more attractive than either water with straw or food; nor were different background effective as found by others. These studies show that the ovipositing female does have some choice on the selection of breeding water, though the laboratory results are conflicting.

Laboratory experiments in enclosed cages suffer from a few serious drawbacks. First, females are forced to lay eggs, even if the water is unsuitable. Secondly, the gradients of humidity, temperatures, odours, etc., get rather blurred in the enclosed space. Nevertheless they can yield useful information if the results are cautiously interpreted.

A. minimus is a species whose egg laying habits have been well studied. Muirhead Thomson (1940a) showed that this species showed a strong preference to ovipositing in shade but paradoxically larvae were scarce in shaded parts of streams. The scarcity was due to the disappearance of vegetation under shade and the consequent increase in water velocity.

That some species show a preference to breeding in waters with slight current has been well documented, as in the case of, *A. fluviatilis* and *A. minimus*. Critical experiments on whether this is due to egg laying preference or to larval behaviour are not available. Muirhead Thomson (1940b) showed that actually the gravid female preferred to lay eggs in still water rather than water with a low current. The explanation given for the presence of larvae only in streams with current was, that the larvae actually were present in side pockets, or in grassy margins, where the current was negligible. The presence of ripples on surface of water did not affect egg laying.

The present author, in certain unpublished studies, had found that when the eggs of *A. fluviatilis* were artificially introduced in the open unshaded parts of a channel, the larvae after hatching tended to move into the grassy parts or under shade (see under *A. fluviatilis*).

Muirhead Thomson had also shown that even *A. minimus* scatters its eggs in many types of breeding places but it disappears in unfavourable places, such as shallow pits etc., in which the temperature exceeds 40°C (see under *A. minimus*). There was also some degree of avoidance of egg laying in high temperatures in the laboratory (Muirhead Thomson, 1940c) but no preferences were exhibited when given a choice of temperatures such as those likely to be seen at night when the actual egg laying occurred.

The time of egg laying is very important when trying to understand the influence of other factors on egg laying in different types of habitats. Russell and Ramachandra Rao (1942e) found that though *A. culicifacies* laid eggs throughout night the greatest number was laid in the first third of the night under natural field conditions. Pal (1945b) in his laboratory experiments in northern India and Muirhead Thomson (1940a & b) with *A. minimus* in Assam, have also noted a similar habit

of early egg laying.

In relation to egg laying by *A. fluviatilis* and *A. minimus* and perhaps other species, it should be borne in mind that if the female exercises any choice, it is at the time of egg laying. The present author made a study (unpublished) in North Kanara District. He found that though there were wide variations in the temperatures during day time, particularly after mid day, the temperatures of the natural breeding places were more or less uniform during the earlier part of the night, when maximum egg laying takes place. Perhaps in nature temperature is not such an important factor as is generally thought in influencing egg laying.

The method of egg laying by mosquitoes which breed in tree holes has not been studied with Indian anophelines. Obviously, the factors involved are very different from those in open waters.

The whole field of oviposition mechanism as well as preferences is an extremely useful subject for study which can explain many anomalies and paradoxes in the breeding habits noticed in nature.

(d) Biting and feeding

Anopheline mosquitoes, except for a few species occurring in the deep shade of the forest, take their blood meals at night time. They feed on man, cattle and other domestic animals, the animals of the forest including monkeys and perhaps birds. Some culicines are known to feed on reptiles and amphibians, but such a habit is not known among any Indian anopheline. Many investigations have been made on the time of feeding and there are considerable variations in the recorded observations. Some species bite throughout the night but with peaks at certain times. Some are exclusively crepuscular. A difference has to be made between entry into a hut or cattleshed and the actual time of biting, though one can presume that biting occurs as early as possible after entry.

A. culicifacies seems to bite mostly in the earlier part of the night prior midnight though some degree of biting continues throughout the night. The report by Viswanathan, Ramachandra Rao and Halgeri (1955), on the basis of critical studies made over a period of about 100 nights near Pune, perhaps exemplifies the typical behaviour of that species. They constructed special huts in which careful observations could be made and all inner surfaces could be searched with ease. A calf was tethered at night in the hut. They found that over 67 per cent of the females entered and took blood meals before midnight. Similar observations, though not on the same scale, have been made by a few other workers in India.

In the observation of Viswanathan and Ramachandra Rao (Viswanathan *et al.*, 1943, 1944) *A. fluviatilis* also was found to bite mostly during the first quarter of the night in North Kanara District. This observation somewhat differs from that made by the workers in Old Mysore State about 200 kms south in the same Western Ghats [Brooke Worth (1953), Bhombore *et al.* (1956)]. The latter found the biting to occur much later in the night. While there can be real differences in the biting rhythms because of existence of biological strains, they could also be due to the methods of work adopted. In critical studies it is essential to collect to depletion

all the adults resting before commencement of the studies on any night and also later at each succeeding periods of searches. Otherwise, there would be accumulations of mosquitoes from previous periods likely to result in fallacious observations.

As shown by Reisen and Aslamkhan (1978) in their very thorough studies near Lahore, there are innate differences in the biting rhythms among mosquitoes, but season also brings about marked variations. In their collection of 18,873 mosquitoes of 18 species of 4 genera over a period of one year in 1976, *Culex tritaeniorhynchus* was the most abundant species (47.3 per cent). Among anophelines, *A. pulcherrimus* formed 14.8 per cent, *A. annularis* 13.6 per cent and *A. culicifacies* 9.2 per cent, *A. nigerrimus* and *A. stephensi* formed about 1.3 per cent and 3.4 per cent respectively. The actual numbers collected were *A. pulcherrimus* 2,789, *A. culicifacies* 1,736, *A. annularis* 2,568, *A. nigerrimus* 267 and *A. stephensi* 643.

Briefly the biting rhythms of these species were:

A. culicifacies	Cooler months (November to March)	Biting took place mostly in the first segment of the night.
	Hot months April and May (September and October)	Biting shifted to 2nd and 3rd segments of the night.
	Mid-summer (June, July and August)	Biting was entirely arhythmic and occurred throughout night.
A. annularis	Throughout the year	1st and 2nd segment of the night, but a slight shift occurred covering a little earlier period, during cold weather.
	[Differring from observations in Bangladesh by Quraishi (1963) and Venkat Rao (1961) in Orissa, who found the peak occurring in 2nd and 3rd quarters of the night.]	
A. nigerrimus	Biting occurs early in the first segment of the night. There is an abruptness of biting during dusk. No seasonal changes have been noticed.	
A. pulcherrimus	Feeds typically prior to midnight.	
A. stephensi	Feeds mostly before midnight, being markedly crepuscular in periods of low ambient temperature.	
A. subpictus	Disappears in the cold period in the Punjab, but is common in post-monsoon months. Feeds mostly prior to midnight with a pre-dawn peak.	

These collections were all made outdoors on cattle baits. Reisen and Aslamkhan (*loc. cit.*) have also made interesting observations on many common culicines which are beyond the scope of this work.

The studies of Reisen and Aslamkhan (1978) were made at five different times of night.

1. Dusk to 20.30 hours
2. 21.00 to 22.00 hours

3. 23.00 to 01.00 hours
4. 02.00 to 03.00 hours
5. 03.00 hours to dawn

They found that under the environmental conditions in the area mosquitoes could be grouped according to the shape of their biting curves:

1. Unimodal — *A. pulcherrimus* and usually *A. culicifacies* and *A. stephensi.*
2. Mostly unimodal with a peak early in the evening but occasionally with a slight increase during the 4th or 5th segment of the night, e.g. *A. annularis* and *A. nigerrimus.*
3. Bimodal with predominant peak mainly in the evening and well defined predawn or dawn peak, e.g. *A. subpictus.*
4. Occasional multimodal or arhythmic, e.g. *A. culicifacies, A. stephensi* and *A. subpictus.*

Reisen and Aslamkhan (1978) postulated the possibility of genetic factors which could influence behaviour. They quoted instances of changes in the degree of anthropophilism and endophagy among different populations in *A. gambiae* complex being related to selected chromosomal polymorphisms occurring mostly in chromosome-2R (White, 1974a, Colluzi *et al.*, 1975). Similar genetic cause could explain the seasonal shift in the degree of anthropophilism and endophily of two species of *Aedes* in the Changa Manga National Forest in Pakistan. They also quote laboratory studies on variation in adult emergence (Colluzi *et al.*, 1972) and flight activity rhythms (Jones, 1974) of *A. stephensi* being related to chromosomal polymorphisms. There is also a possibility of the environment and season playing an important role in the pattern of biting activity and many of the differences in the observations made by Indian authors could as well be due to this factor.

Some of the observations made in other species could briefly be mentioned.

A. philippinensis: Observations have been made mostly in other countries of Southeast Asia, particularly in Malaysia. Reid and Wharton (1949) found the feeding to commence at about 21.00 hours and to go on throughout the night. In Burma, Mecan had found peak biting activity between 22.00 to 23.00 hours and again between 01.00 to 01.30 hours. In Burnihat, Assam/Meghalaya, Rajagopal (1976) found this species to feed on man all through the night from 18.30 hours to 04.00 hours with peak feeding between 19.00 and 21.00 hours and again from 01.00 to 04.00 hours.

A. stephensi is largely a nocturnal feeder. Nursing *et al.* (1934) in Old Mysore found a bimodal rhythm, one peak between 21.00 and 24.00 hours and another between 04.00 to 06.00 hours. The species could also bite during daytime even at 09.00 hours and also in the evenings in the open as observed by Deburca and Jacob (1947).

A. sundaicus: According to Venkat Rao (1961a) the biting takes place in the first half of the night rather than in the second half. When occurring in large numbers the species bites throughout the night and some specimens have been seen to feed

even in early daylight hours. In Indonesia, Sundararaman *et al.* (1957) observed that most of the feeding occurred during the second and third quarters of the night.

A. minimus: This species appears to be a late biter. Muirhead Thomson (1941b) and Krishnaswamy (1952) have both observed that bulk of the feeding takes place in the third and fourth quarters of the night. In Thailand it has been found to be an early biter during the dry season and a late biter during the wet season.

A. varuna: Senior White *et al.* (1945) found that along with other members of the *fluviatilis* group feeding was mainly between midnight and 05.00 hours.

A. jeyporiensis var. *candidiensis:* In Old Mysore State, Nursing *et al.* (1934) found more specimens between 04.00 to 06.00 hours than between 21.00 to 24.00 hours (54.0 and 46.0 per cent respectively). In Burma, Mecan found freshly fed specimens between 21.00 to 21.30 hours, but in many houses the main feeding was between 22.00 hours and 03.00 hours. Smoke in the house seemed to delay the entry of the mosquitoes.

A. balabacensis is generally regarded as a late biter during most parts of the year but with a tendency to bite earlier in the dry season (Ismail *et al.*, 1974). In observations of Colless and others, in Thailand *A. leucosphyrus* has been found also to be a late night biter.

Some very interesting comparative observations have been made on a few Malaysian mosquitoes regarding their biting time by Moorhouse and Wharton (1965) on human baits.

A. umbrosus	The main biting time was between 17.00 and 18.00 hours and again at 07.00 hours, i.e., when there was broad daylight. More biting took place during daylight hours than at night.
A. letifer	Biting occurred mostly throughout night with a peak at about 23.00 hours. Negligible biting during daytime.
A. roperi	Biting occurred only during daytime with a peak before dusk at 17.00 hours.
A. donaldi	Biting occurred throughout day and night, the day biting being a little higher than the night biting.

These data are very interesting in bringing to light the fact that there are innate differences even between closely related species.

To sum up, females of anophelines of India bite mainly at night but occasional observations have been made of biting during daylight hours also. The species differ considerably in their biting rhythms. Though biting may occur throughout night on a small scale, there are distinct peak times. Some species bite early and others bite late. There are innate differences between the species but the behaviour of the same species can be influenced by seasonal factors also.

The biting cycle or rhythm of each species is dealt with in more detail under the particular species.

(e) Flight and dispersal

Flight and dispersal characteristics of anophelines have an important bering on malaria epidemiology. Not only do they have relation to the intensity and spread of the disease, but also to the kind and extent of control measures required to reduce or interrupt transmission.

It is necessary to distinguish between flight and dispersal. Flight is the travel which anophelines perform in single flights while on the wing and dispersal is the spreading out of mosquitoes by repeated short flights in the course of their life time. The words have, however, been used rather indiscriminately. Sometimes the word "infiltration" has also been used. When we say that the adults of a species are found 1.5 km away from the place of release, all we mean is that at the end of a certain period of time, the adults are found at that distance. It would not be certain whether they covered the distance in one effort when on the wing or by a series of shorter flights, after coming to rest on the ground for brief periods. Therefore, when expressions such as "flight range", "maximum flight distance", "effective flight range", etc. are used, they apply only to the end result, i.e., total distance travelled and not neccessarily to the physical ability of a mosquito to fly that distance in one effort.

Flight and dispersal are controlled not only by the innate abilities of the mosquitoes but also by the nature of the evironment. The concentration or dispersal of the sources of blood meal, the availability and spread of the preferred breeding places, the prevalence of the physical barriers such as large trees as in forests, high hills, large stretches of water and wind velocity, etc. modify the flight and dispersal patterns.

Generally speaking, mosquitoes are required to perform flights for several purposes, such as for sexual activities including swarming and mating (called epigamic behaviour), for seeking hosts on which to feed, for seeking breeding places for egg laying and for seeking suitable day time shelters. At nights, when most anopheline species are active, they wander about quite a lot being attracted hither and thither by various stimuli leading sometimes to what appears to man as purposeless flight.

The physical and biological stimuli which influence mosquitoes to take to wings and the direction of flight are not adequately understood to permit a reliable explanation of various types of locomotor behaviour. There are inner physiological processes which regulate the direction of flight. The same individual female flies towards breeding places for oviposition when it is gravid, while after egg laying its direction is towards hosts for feeding. In the early dawn the mosquitoes fly towards dark places. These could all be explained only when critical studies on the orientation mechanisms are carried out.

Since the time of James and Liston (1911), half a mile (0.8 kms) has been regarded as the normal flight range of the major species of Indian anophelines. This was based on the detection of adults at points from the nearest larval sources and observations on effectiveness of control when carried out to various distances and rarely on direct observations. This conclusion, though not absolute, has been found to be generally applicable to the Indian anophelines.

Critical studies on flight and dispersal have been made only with a few major vector species.

A. culicifacies has received most attention. Afridi *et al.* (1940) in Karnal, Haryana, found that the females of the species could fly about 1500 feet (460 m). But their studies were all made by recapturing mosquitoes mainly in one direction. Russell *et al.* (1944), who made a large experiment in which they released over 50,000 *A. culicifacies* adults of both sexes in southern India, were able to study the flight habits in considerable detail. The flights in all directions were studied by providing 80 trap huts uniformly spread all over a radius of one mile in four concentric circles. Each hut had a cow-calf tethered at night. It was found that while individual specimens could fly upto 1.75 miles (2.8 km) in one night, many were concentrated closer to the point of release. They found that the dispersal noticed in the first two days after release of marked mosquitoes followed a certain pattern. The relationship between numbers and distance travelled was hyperbolic and the data showed that the numbers recaptured at various concentric circles diminished directly with the square of the distance. However, the average distance i.e., the distance upto which 50 per cent of the adults travelled, was only a little over ¼ of a mile (400 m). The distance upto which control of larvae was necessary to protect a village varies with the initial densities present in it. In the Pattukkottai area, it appeared to be about 0.75 miles (1.2 km) to reduce a density of 50 per manhour to 5 per manhour. If the initial density was only 10 per manhour the reduction required would be only 50% and a distance of about 0.5 miles (0.8 kms) would be adequate.

Regarding *A. fluviatilis* there is general agreement that they do not fly far away from the places of feeding if suitable breeding places occur closeby. The work of Kerala Workers in Wynaad showed that effective flight range would be hardly 1,000 feet (304 m). There are also other observations to show that the breeding of the species is concentrated in breeding places very close to the villages.

A. stephensi in urban area is regarded to have a low flight range, but critical observations are not available. However, in rural areas it can fly upto a distance of about 3.5 miles (5.2 kms) (Deburca, 1946). Observations in Bahrein (Persian Gulf) have shown that the adults were found resting 2.4 kms away from the closest breeding place. In an experimental study in Iran it has been found that *A. stephensi* var. *mysorensis* P^{32} tagged specimens, released from central points, could be recaptured upto a distance of 4.5 kms.

Practically, no reliable information exists on such vector species as *A. balabacensis**, *A. philippinensis* and *A. varuna* in India.

In *A. annularis*, however, the work of Venkat Rao has shown that swarms of the species occurred very close to the Chilka Lake where the species was breeding while those of A. *sundaicus* and *A. subpictus* were found 3.2 kms away indicating a much higher flight range of the latter two species. *A. sundaicus* has a much greater disper-

*A. *balabacensis* has been found in other Southeast Asian countries to fly upto 0.8 kms and the range of distribution could be upto more than 1.6 kms.

sal range than many other species. Observations by Covell and Pritam Singh (1942) showed that the adults were present 9.6 kms away from the shore of the Chilka Lake. In the Andamans, a dispersal of over 1.6 kms has been recorded. Venkat Rao *et al.*'s observations (1942) confirmed that the adults of this species can traverse a distance of about 2.4 kms for swarming.

In malaria epidemiology, it is not the "total flight range", i.e., total distance upto which an individual or two may fly, which is important. What matters is the "effective flight range" which can be defined as the distance from which adequate numbers can fly into a central point to build up densities of the vector necessary for malaria transmission. Effective flight range can vary considerably according to the terrain and also on the distribution of houses and cattle sheds in the area. If a species can get suitable hosts for feeding, appropriate breeding places for egg laying, and adequate opportunities for daytime shelter, all close together, there would seem to be no need for long flights. On the other hand, if the only source for blood meals happens to be even 2 to 3 kms away from a breeding place, anophelines could fly that distance to satisfy their hunger. An example is of *A. culicifacies* in Fort Sandaman (Pakistan) where Deburca (1939) found *A. culicifacies* adults in numbers when the only breeding place, a river, was about 5.2 kms away. There is a reference to *A.pulcherrimus* adults being collected when flying on a ship 25 kms out in the open sea near Shatt-el-Arab (Wright, 1917).

Tall trees can provide obstructions to flights, as may be seen from the observation of Covell on *A. sundaicus*. Covell even recommended prevention of cutting the belt of trees, a potential means of malaria prevention.

Males have a much shorter flight range than females. In the Pattukkottai experiments, males of *A. culicifacies* were also found to fly distance of over one mile in one night, but their numbers were small. As compared to females, there was greater recapture of males at shorter distances. It is a general observation that the presence of a large number of males in a resting place during daytime is indicative of breeding places nearby.

The Pattukkottai experiment also showed that *A. culicifacies* instead of being wafted with the wind, dispersed equally in all directions and even that more adults flew against wind up to one quarter mile than in the other quadrants. However, there is evidence to suggest that anophelines can also be wafted by air currents. Russell had found that many cases of malaria had occurred in a boarding school at Keti near Ootacamund [elevation approximately 7,000 feet (2100 m)]. There was absolutely no breeding of *A. fluviatilis*, the vector, in the locality. The Keti is situated at the top of steep valley and the nearest breeding places of *A.fluviatilis* were 1000-1500 feet (300-450 m) below. The inmates of the boarding school had not gone down to the endemic areas down below at the appropriate time. It was surmised that infected *A. fluviatilis* females had been wafted by air currents up the valley.

Gross migratory flights as noted with some European and American anophelines, have not been recorded with Indian species so far. In those countries, flights upto and over 100 kms are known.

(f) Shelter seeking at dawn

During night when mosquitoes are in their active phase they are attracted hither and thither by various kinds of stimuli. They are quite alert during night and they are responsive to stimuli very readily. Even a beam of light from a torch light, which does not affect them during daytime, disturbs them at night and they start flying.

As the work of Jaswant Singh and Mohan (1951) on *A. fluviatilis* has shown, the adults are frequently moving from place to place and even leave the premises in which they have fed. Some come back but others stay out. Some of the movements appear to us to be purposeless flights but may have an evolutionary significance connected with survival.

When the twilight of the dawn comes, there is a kind of disturbance in mosquitoes which are in the open. They become restless and begin to fly about simulating swarming activities though not on the same scale as in the evenings. The present author has actually seen several swarms of *Culex fatigans* at this time and many of May-flies (Ephemeroptera). Experimentally such movements have been seen by him in *Cules molestus* and *Anopheles maculipennis*. Probably such activities occur within anophelines also in nature.

Whether such swarming activities occur or not at dawn, the anophelines are on flight. As the light intensity increases the anophelines are repelled by strong light (negative phototaxy to strong light) and fly towards any dark objects which come into their field of vision. A dark area, a bush, a tree or a hole, becomes their target. In forests and wooden areas, bushes and such places are the only places they can reach, but in urban areas, it is the dark windows, doors, caves, etc., towards which they fly. They enter such places and as light becomes stronger they go deeper into the shelters. When the light is really strong, they begin to become inactive, presumably because of the depressant effect of strong light and also perhaps partly due to a diurnal rhythm. They stay in such shelters till the evening.

Therefore, apart from a proportion of the population which had remained in a hut or cattle shed during the night, many enter such shelters during dawn. The entry into a hut or into a bush depends on the environment, urban or sylvan, with various intermediate grades between them. Shelter seeking occurs at dawn and the environment has a great influence on it. The same species can be an indoor-rester in a town but an outdoor-rester in a garden or forest. Therefore, the distinction between endophily or exophily is rather artificial.

It is well known that such species as *A. balabacensis* and *A. philippinensis* in forest areas, even though they may enter dwellings for feeding, leave the shelters before dawn and do not return. Even *A. culicifacies* in certain forested areas stay outside. This can be attributed to the environment rather than to the species itself.

One, however, cannot entirely rule out differences between certain species. Some may be more active at night and therefore fly out much more than the others. While good proportions of *A. culicifacies* rest indoors after feeding, very few *A. nigerrimus* do so in the same locality. This can be attributed to certain innate differences. If a species has a tendency to fly out more at night, it is taken away from human habitations and therefore, is less likely to re-enter houses. There are numerous

examples of such differences and it would be a worthwhile study to approach the problem of daytime resting habits on the basis of critical experiments and observations using the modern techniques of sensory physiology. The classic work on the Orientation of Animals by Fraenkel and Gunn (1940) and the book on Natural History of Mosquitoes by Bates (1949) are worth studying.

The movement towards dark by disturbed mosquitoes are well illustrated by the experiments of Kennedy (1940) on *Aedes aegypti* and of Ramachandra Rao (1947) on *A. maculipennis* and *Culex molestus*. Kennedy suspended the mosquitoes by silken threads and when a dark stripe was exposed to them they invariably turned towards it. Ramachandra Rao used mosquitoes which had been artificially rendered flightless by tying the wings together with horse hair. In a uniform arena lighted from below, the mosquitoes always moved towards dark stripes presented to them. If two stripes were presented, they went more towards the wider one. When the mosquito was slowly walking aimlessly and a dark stripe suddenly appeared in its field of vision, it rapidly turned towards it. If one of the eyes was blinded by Indian ink, the mosquito performed circus movements continuously turning towards the side of the blinded eye. These experiments were done on *Culex molestus* and *A. maculipennis*.

All these experimental observations showed that dark objects were attractive (actually this is negative phototaxy to strong light as there can be no positive taxis towards darkness). During evenings, it was found that the free mosquitoes as well as mosquitoes which had been rendered flightless actually moved towards the still lighted windows. Though these experiments were not carried out with anophelines, except *A. maculipennis* to a little extent, they give pointers to the habits of mosquitoes in general. It is to be recognised that one cannot draw general conclusions without specific work on Indian anophelines. This is a rather neglected field of study, and many erroneous statements are on record regarding behaviour of anophelines based on human interpretation of the activities.

Because of the inward and outward movements of anophelines every night, the adult populations in shelters is not composed entirely of the same individuals. There would be some which would have remained from the previous nights, but others would have come in, unfed, fed or semi-gravid. This has been termed as the 'daily turnover' the existence of which had been recognised for quite some time.

Often the existence of a "homing instinct" has been attributed to anophelines suggesting that some individuals come to the same house or cattle sheds repeatedly. There is no evidence for this. However, in the experiments of Viswanathan, Ramachandra Rao and Rama Rao (1944) with *A. fluviatilis*, individuals which had been released in the experimental tents had returned to the same place after oviposition. This can be attributed to the fact that the breeding place was very closeby and the gravid females had gone out to lay eggs and returned to the tent which was the closest structure with human hosts. In small villages or isolated structures such a phenomenon is possible. But there is no evidence that it occurs to any considerable extent in larger villages with species which have a wide range of dispersal.

This chapter on the daily life of anopheline cannot have a better epilogue than

the statement made by Samuel Taylor Darling that malariologists who study the habits of the mosquitoes should "begin to think like mosquitoes". Interpreting the observations on mosquitoes from purely a human point of view is likely to lead to fallacious conclusions.

3. Host Preferences

Anopheline females are known to feed on the blood of a wide variety of animals including man, monkeys, cattle, horses, dogs, pigs, camels, other ruminants, birds, etc. Some specimens show distinct preferences in this regard, while others are facultative and feed on any animal which comes along. Only the species which prefer to bite man, in large numbers, can be important vectors of human diseases. The exact orientation mechanisms which direct the anophelines to man or other hosts is not fully understood. Odours, colour, CO_2, temperature and humidity gradients, etc., have all been suggested. But it is very well known that in the absence of the preferred host, anopheline females will not be reluctant to take blood meals on other animals. The host preference is an important factor in malaria epidemiology.

Bruce-Chwatt, *et al.* (1966), whose comprehensive paper should be read by all malariologists, made a distinction between 'host selection' and 'host preference'. 'Host selection' indicates the host actually fed upon and 'host preference' indicates the innate habit of exercising choice of a host or hosts. The latter can be assessed only by an experimental approach, as for example, when females are given a free choice of hosts in a large enclosed space. It is well known that a few species prefer to feed on man, if available, but will also feed on other animals if a human host is not available.

There are several ways by means of which the host preference of particular species can be determined.

(1) One of the commonest methods which till recently had been used was to find out the proportion of resting in human dwellings and cattle sheds. It used to be presumed that the resting place was also by and large the feeding place and therefore those which rested in houses were considered largely to have bitten man and those in cattle sheds the cattle. It is now recognised that the place of shelter is not necessarily the place of feeding. However, in the data reported by Bruce-Chwatt *et al. (loc. cit.)* generally higher proportions of human biting individuals have been found in houses than in other biotopes.

(2) A more reliable method is to carry out what are known as precipitin tests. Bloods in the stomachs of freshly fed mosquitoes are pressed on to filter papers and allowed to dry. In the laboratory, they are tested against antisera for bloods of man and various kinds of animals. When the stomach blood of a mosquito reacts against antiserum of any host there is presumptive evidence that the blood in the stomach belongs to that host. Precipitin tests using refined techniques were adopted for rapid examination of large numbers of mosquito stomach bloods. The one perfected by Rice and Barber (1935) has been in very wide use. With the development of still better techniques (Weitz, 1956), precipitin tests have been standardized and

used against mosquito bloods from all parts of the world not only by individuals, but by national laboratories and selected laboratories of the World Health Organisation. Details of the methods of the tests will not be given here, but reference can be made to an excellent publication by W.H.O. (1975b).

The percentage of stomach bloods which show positive reaction to human antisera is generally regarded as the Anthropophilic Index (AI). A more meaningful term called the 'human blood index' (HBI) has been adopted by the WHO (1963). It is defined as 'the proportion of freshly fed *Anopheles* giving a positive precipitin reaction for human blood in the particular conditions in which capture was made'. The term zoophilic index is the percentage of stomach bloods which show reaction to antisera of animals. However, as the type and number of animals tested vary considerably and the number of antisera is usually of limited types, such an index would not precisely show whether the blood was one of cattle, horses, donkeys, camels, dogs, pigs, deer, sheep, goats, birds, etc. There would be many negative reactions when only the sera of man and cattle are employed. In normal malariological studies in villages, however, zoophilic index may be taken to refer mainly to the percentage found to have bitten domestic cattle. The results of these tests so far as Indian anophelines are concerned, are given in the appropriate sections relating to the individual species.

Over the years many tests have been carried out in India and broadly speaking only four Indian anophelines are known to have high anthropophilic index (AI). They are *A. fluviatilis, A. minimus, A. balabacensis,* and *A. varuna* in which AIs of over 60-70 per cent are common. Most of the other species have predominantly high animal biting indices and low AIs. In some species under certain circumstances a high AI may be found because of unusual environmental conditions such as the absence or scarcity of animals.

There are numerous references to the results of precipitin tests made in India. Given in Table 3 is a list of selected and representative studies.

Table 3. Anthropophilic indices of Indian anophelines

	A.I. %	*Locality*	*Authors*
A. culicifacies			
Low rates	9.9	Delhi	Afridi *et al.* (1938)
	1.9	Pune	Barber and Rice (1938)
	2.5	Pattukkottai	Russell *et al.* (1938)
	12.9	East Central India	Senior White (1947)
High rates	22.3	Delhi epidemics	Covell and Jaswant Singh (1943)
	47.3	Assam and North Bengal	Ramsay *et al.* (1936)
	80.0	Ennore, Tamil Nadu	Russell and Jacob (1939a)
*A. balabacensis**	75.0	North Bengal, Assam	Ramsay *et al.* (1936)
A. jeyporiensis var. *candidiensis*	Very few tests in India, but highly anthropophilic in other Southeast Asian countries.		
A. annularis	1.3	Orissa	Senior White (1947)
	1.8	Madhya Pradesh	
A. philippinensis	6.1	Assam	Ramsay and Macdonald (1936)

(Continued)

*This species has still higher AI rates in certain Southeast Asian countries.

	A.I. %	Locality	Authors
A. stephensi			
Low rates	3.4	Bengal	Roy *et al.* (1938)
	1.4	Punjab	Afridi *et al.* (1939)
	0.8	Bellary, Karnataka	Bhaskar Rao *et al.* (1946)
	11.0	Bellary	Malaria Institute of India Reported
High rates	41.0	Munirabad, Karnataka	Krishnan (1961)
	47.0	Ajmer, Rajasthan	
*A. sundaicus***	5.6	North Vishakhapatnam	Senior White (1947)
A. fluviatilis			
High rates	97.0	Wynaad, Kerala	Covell and Harbhagwan (1939)
	63.6	North Kanara	Jaswant Singh and Jacob (1944)
	56.8	East Central India	Senior White (1947)
Low rates	4.6	Pune	Barber and Rice (1938)
	3.8	Assam	Ramsay *et al.* (1936)
A. minimus	85.7	Assam	Ramsay *et. al.* (1936)
	92.4	Jeypore Hill Tracts	Senior White (1947)
A. varuna	63.1 to 85.7	Jeypore Hills	
	9.1 to 58.5	Vishakhapatnam	Venkat Rao (1961b)
	4.0 to 53.2	Singhbhum Hills	

Among major vectors, those which have low AIs are:

A. culicifacies — The AIs have ranged generally between 2 to 30 per cent. In an unusual situation in a locality north of Madras city a positive index of 80 per cent was found due to scarcity of cattle. There is also evidence of more feeding on man during epidemics.

A. stephensi — AIs have ranged from 1.0 per cent sometimes upto 47 per cent. The type form is more anthropophilic than var. *mysorensis*. The variety *mysorensis* is predominantly zoophilic.

A. philippinensis — AIs about 6.5 per cent.

A. annularis
A. sundaicus — AIs 1 to 2 per cent.
In North Vishakhapatnam in Andhra Pradesh an AI of 5.6 per cent has been found. Very few other observations have been made in India, but in Indonesia it strongly preferred to feed on man with an AI between 22 and 86 per cent.

One species, *A. elegans*, is known to feed exclusively on monkeys.

An extensive review of the host selection by anopheline mosquitoes was made by Bruce Chwatt *et al.* (1966). Apart from discussion of the general numbers tested and interpretation of positive tests, including the fallacies which may arise, they reviewed the human biting indices (HBI) of anophelines all over the world.

** This species shows much higher AI's in other Southeast Asian countries, ranging upto 94 per cent. See Venkat Rao (1961) and Reid (1968).

They pointed out that several species, such as *A. leucosphyrus, A. hackeri* and *A. letifer* of Southeast Asia, may feed both on man and simian hosts. Consolidating the results of nearly 10 years work at the NICD, Delhi and of the Lister Institute, London, they reported HBI of many species and compared the data for India and neighbouring countries such as Nepal and Sri Lanka (Table 4).

Table 4. Human blood indices of some malaria vectors

	HBI and size of sample	
	India	*Nepal*
A. culicifacies	0.040 (2622)	0.189 (3688)
A. fluviatilis	0.027 (1372)	0.184 (3309)
A. stephensi	0.018 (355)	—
A. sundaicus	0.07 (127)	—
A. minimus	—	0.600 (2511)
A. annularis	0.22 (672)	0.026 (1991)

Note: A HBI of 0.1 is about equal to an anthropophilic index of 10 per cent. The HBI is expressed as a proportion and not as a percentage.

With reference to *A. culicifacies* and *A. fluviatilis* it can be noted that a much lower degree of contact with man occurs in India than in Nepal. Whether such a reduction would be caused by increased outdoor resting by DDT irritated mosquitoes surviving in the sprayed area is a matter for speculation. If DDT pressure had produced real changes. this must represent changes of host selection and also may reflect changes of host preference.

Further, these data showed in *A. minimus* an HBI equal to 0.60 in Nepal while Senior White found an HBI of 0.61 in East Central India.

The results of the precipitin tests made on *A. fluviatilis* by Senior White in East Central India and by Issaris *et al.* in the U.P. Terai are shown in Table 5.

Table 5. Human blood indices of *A. fluviatilis*: comparison

Biotope	East Central India (1947)		U.P. Terai (1949-52)	
	No. of smears	*Per cent with human blood*	*No. of smears*	*Per cent with human blood*
Cattle sheds	445	11.7	134	28.4
Outdoors	39	74.4	38	44.7
Human dwellings	1050	56.8	73	63.0
Total	1534	44.1/36.8*	245	42.2/47.5*

*Weighted means, as used by the authors cited, followed by unweighted means.

According to the weighted means, the HBIs are 0.368 in East Central India and 0.475 in U.P. Terai.

Some pertinent information which Bruce-Chwatt *et al.* provide in regard to Indian species on tests carried out between 1955 and 1964 is extracted in Table 6.

Table 6. Comparative Human blood indices of Anophelines:

Species	*Total blood smears*	*Total positive tests*	*Positive for primate blood*		*Countries*
			No.	*Per cent*	
A. aconitus	112	109	5	4.8	Nepal
A. annularis	802	629	2	0.3	India, Indonesia
	2934	2741	182	6.6	Vietnam, India Sri Lanka, Nepal
A. barbirostris	120	118	0	—	India
A. culicifacies	721	712	223	32.7	Sri Lanka,
			489	0.0	(Unsprayed area) India, (mainly DDT sprayed area)
	6830	6432	198	3.1	India, Nepal, Sri Lanka
A. elegans	130	128	126	98.4*	India, Madras
A. fluviatilis	75	58	19	32.8	Iran, Iraq, Saudi Arabia
	526	519	3	0.6	India, Nepal, Pakistan, Saudi Arabia
	5035	4751	678	14.3	India, Nepal, Sri Lanka
A. jeyporiensis s. l.	12	12	0	—	Pakistan, Vietnam
	244	237	20	8.4	India, Nepal
A. maculatus	2567	2432	55	2.3	India, Sri Lanka Nepal
A. philippinensis	111	111	2	1.8	India
A. splendidus	914	865	12	1.4	Nepal, India
A. subpictus	2795	2607	241	9.2	India, Nepal, Sri Lanka
A. sundaicus	160	127	9	7.1	India, West Bengal
A. tessellatus	403	360	4	1.1	India
A. vagus s. l.	464	425	30	6.5	India, Nepal, Sri Lanka
A. varuna	226	216	59	27.3	India, Nepal

*This refers to monkey blood and not to human blood.

Note: They used the word 'primate blood' instead of human blood. However in one case where the primate blood referred to monkey blood, it is so indicated (*A. elegans*). Therefore, all figures except for *A. elegans* can be taken to refer to human blood.

In a series of precipitin tests carried out by the N.I.C.D., Delhi of samples from India, Sri Lanka and Nepal between 1960 and 1961, the percentage of positive for man are shown in Table 7.

Table 7. Comparative AI's of Sri Lanka, Nepal and India

Species	Sri Lanka		Nepal		India	
	No. of tests	% positive for primate blood	No. of tests	% positive for human blood	No. of tests	% positive for primate blood
A. annularis	—	—	2113	H 4.0 O 1.1	120	0.0
A. culicifacies	116	37.8	3906	H 36.7 O 1.1	2808	2.5
A. fluviatilis	75	7.8	3529	H 34.5 O 2.3	1431	4.8 0.8
A. hyrcanus s. l.	52	—	102	H 3.6 O —	338	0.0
A. jeyporiensis s. l.	—	—	40	—	204	—
A. maculatus s. l.	72	—	2334	H 10.9 O 1.0		
A. minimus		4.9	2625	H 92.9 O 27.0		
A. pallidus	119	13.6				
A. splendidus			817	H 2.2 O 1.1		
A. subpictus	578	13.6 18.8	63	—		
A. vagus	97	2.6	314	1.1		
A. varuna			108	8.0		

(H = Human dwellings, O = Others)

Note: HBI is expressed as a proportion, and 0.1 is approximately equal to 10% anthropophilic index. Conversion from A.I. to H.B.I. requires certain corrections. Therefore A.I. and H.B.I. are not exactly the same.

It can be noted that the AI's were generally higher in mosquitoes collected in human dwellings than in those collected in other places. Also, the AI's of most species were higher in Nepal and Sri Lanka than in India. Bruce-Chwatt *et al.*, state that there is some evidence of changes of host selection in populations of certain species in areas where residual insecticides have been used on a large scale, a positive factor favouring reduction of malaria transmission.

(3) A third method of determining host preferences is to collect mosquitoes directly off the hosts in the actual act of feeding or using traps with selected baits. Some work has been done on this aspect in India, but it is not enough to permit critical evaluations. Several biting collections on man or cattle have been made but comparative evaluation has not been satisfactory. As examples may be cited Sen *et al.* (1973) in Tirap, Ramachandra Rao and Rajagopalan (1957) in Pune, and Rajagopal (1976) in Assam. Such studies indicate whether a species bites man, and if so how many bite him. They cannot give an indication of the comparative attraction

of man and cattle. Reisen *et al.*, have made some comparative observations in Lahore which have been referred to already.

Some more critical studies have been made in other Southeast Asian countries by workers such as Warren, Eyles, Wharton and others. Reid (1968) has consolidated their data and categorized their species by their attraction to man. Given below is a list of species which occur in India in the order of decreasing attraction to man.

1. *A. balabacensis*
2. *A. minimus*
3. *A. maculatus* (Malaya)
4. *A. sundaicus*
5. *A. karwari*
6. *A. aconitus*
7. *A. sinensis*
8. *A. nitidus*
9. "*A. philippinensis*"
10. *A. barbirostris*
11. *A. tessellatus*
12. *A. kochi*
13. *A. vagus*

The first four are important vectors of malaria in Southeast Asia, but *A. philippinensis* may be added to the list of vectors in India. Purely Indian malaria vectors, namely *A. culicifacies, A. fluviatilis, A. annularis* and *A. varuna* do not for obvious reasons, figure in the Southeast Asian list.

The whole subject of host preference (or host selection) is quite complex and the behaviour of mosquitoes varies considerably from place to place depending upon many environmental factors and also because of strain variation in the same species. An excellent example of the strain variation is in respect of *A. fluviatilis* which occurs in two forms, a highly anthropophilic form in the Western Ghats, the Himalayan foothills and in the hills of east central India, and a highly zoophilic form in the Deccan plateau. Why certain species prefer to bite man and others prefer cattle is a problem of extreme interest and may be related to intrinsic genetic factors developed during the course of evolution.

Roubaud (1921) had postulated that the species with more teeth on its maxilla (high maxillary index) could easily bite cattle and only those which have fewer teeth (low maxillary index) could bite man and therefore be vectors. This hypothesis has been shown to be not valid by the work of Senior White (1937b) and Russell and Ramachandra Rao (1942c). Detailed and critical studies on the sensory physiology and orientation mechanisms alone can answer the problem satisfactorily.

4. Gonotrophic Relationship

Only female anophelines take blood meals on appropriate hosts, viz., mammals, birds or other animals. The males do not suck blood but live on vegetable juices. Females as well as males can survive very well in the laboratory by feeding on sugar or glucose solutions or water soaked raisins. It is believed that when females take such juices they go into the oesophageal diverticula rather than into the stomach. But quite often sugar juices have been found in the stomach also. The imbibing of blood by females and egg production are closely related. In anophelines no instance of development of ovaries and maturation of eggs has been observed in the absence of blood meals, unlike in certain species of culicines such as *Culex pipiens molestus* of Europe which can develop one or more batches of eggs without a blood meal.

Though subsequent batches of eggs can develop without blood meals, most species feed on man and animals normally after the first gonotrophic cycle. This habit of developing eggs without blood meals was termed "autogeny" by Roubaud. There are also several species of culicines (for example *Toxorhynchites* spp.) whose proboscis cannot penetrate the skin of animals and which feed and thrive on vegetable juices only, but can still develop eggs.

The frequency of feeding is linked with the digestion of blood and the maturation of eggs and oviposition. The two processes proceed simultaneously; as the blood is digested, eggs are developed. By the time the eggs are developed, the blood is almost completely digested. This is known as the gonotrophical relationship. Usually, except in the first oviposition, the time taken for development of eggs and oviposition is 48 or 72 hours. In warmer weather the common species of anophelines complete the process in 48 hours, but in colder weather it may take 72 hours or sometimes even longer. For the first oviposition the time taken may be 72-96 hours (3-4 days) but the time taken for the second and subsequent ovipositions is shorter. Though the cycle may take 48 or 72 hours some individuals may not be able to take their second and subsequent blood meals on the very night in which they deposit their eggs. Therefore, in a proportion of individuals, the cycle may be prolonged by a day. There may be other individuals which in forced circumstances may not be able to reach the suitable breeding sites at the end of 48 hours. While 48 and 72 hours may be taken as a general rule, there are certain exceptions not only within the same species but even in the same individuals.

Blood meals are taken (*a*) by freshly hatched hungry females (nulliparous), (*b*) by females which have deposited at least one batch of eggs (uniparous) or two or more batches of eggs (multiparous). Though many females may take blood meals on the very night in which they hatch out, perhaps many fail to do so and take the blood meals on the second and third nights because after eclosion, some time is required for the cuticle to harden.

Digestion of blood and the development of ovaries take place simultaneously and one is related to the other. There are many instances, however, in which more than one blood meal is taken by the same individual female before the maturation of eggs. Such multiple feeding within the same gonotrophic cycle is not uncommon. The freshly imbibed blood appears bright and red for a short period while the old blood is dark and opaque. This helps, therefore, to determine whether a mosquito has taken a blood meal recently or some hours earlier. As the hours pass the blood becomes dark and opaque. As the blood is digested and the ovaries develop, the space in the abdomen occupied by the former diminishes and that occupied by the latter increases. By the end of 24 hours normally half of the abdomen contains dark blood and the other half is filled by developing ova. A small streak of pigmented blood remains in the lower part of the abdomen even when the eggs are fully developed. However, if the egg laying is postponed, even this streak of blood on the ventral side disappears.

The study of gonotrophic conditions in anopheline populations at a given time will give some useful information not only on the time taken for the gonotrophical

cycle, but also on the degree of outdoor resting.

Many authors have used abdominal condition for determining the stage of the gonotrophic cycle, but the one which the present author has found practically useful is given below (Fig.1). (see Viswanathan, Ramachandra Rao and Halgeri, 1955).

Class A	Empty abdomen: unfed	A1 with no trace of blood. A2 with traces of previous blood meal.
Class B	Freshly fed	B1 freshly fed, no trace of old blood or of ovarian development. B2 freshly fed, with traces of old blood but no ovarian development. B3 freshly but partially fed with traces of partially digested blood and of ovarian development. B4 partially fed without traces of old blood or of ovarian development (interrupted feeding).
Class C	Partially digested blood and partial ovarian development — semigravid	
Class D	Gravid, eggs fully developed but abdomen with a slight trace of old blood.	
Class E	Gravid, eggs fully developed without trace of old blood in the abdomen.	
Class F	Miscellaneous	F1 unclassified F2 partial digestion of blood, but no ovarian development.

The gonotrophic cycle is regulated by endogenous rhythms apart from the climatic conditions. The rhythm is not of one day's duration but may be of two or three days' duration. This rhythm is sometimes upset and the feeding and ovarian development may not coincide. Swellengrebel (1929) gave it the name of "gonotrophic dissociation".

Gonotrophic dissociation may be described as a lack of association between feeding and egg development and egg laying. The females may continue to feed at regular intervals without ovarian development taking place. This phenomenon can lead to repeated feeding as observed in European mosquitoes in warm houses in

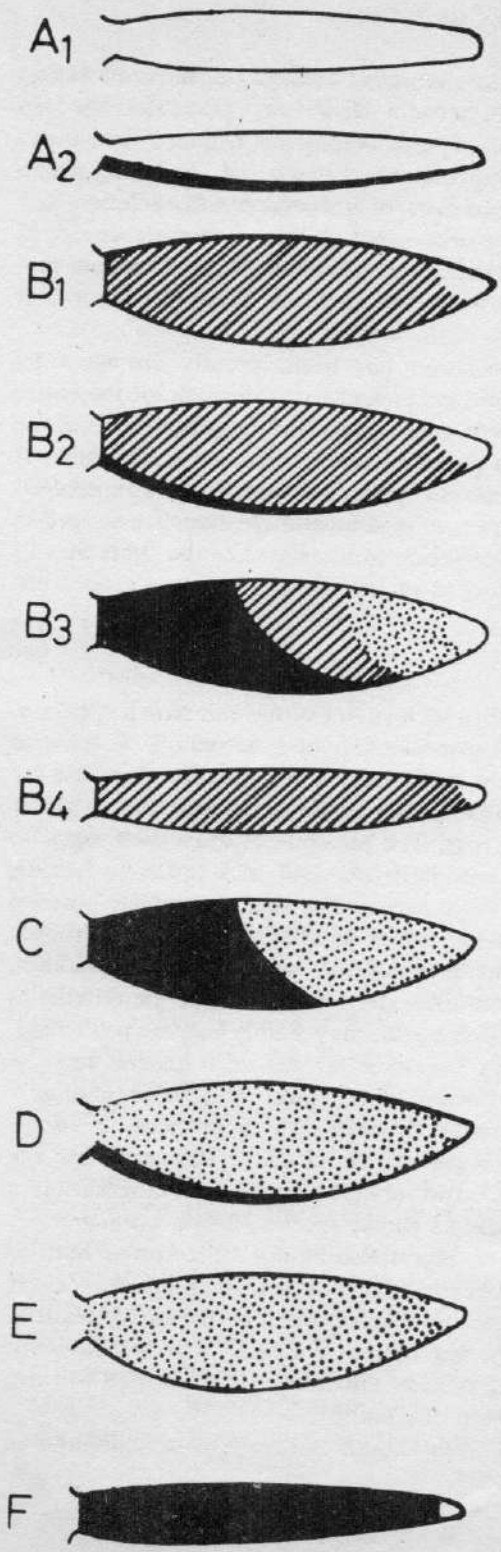


Figure 1. Abdomen of female *Anopheles* adults. Diagrammatic classification of abdominal gonotrophic conditions.

A — Unfed
A 1 — Unfed; freshly hatched
A 2 — Unfed; with traces of previous blood meal.
B — Freshly fed.
B 1 — Freshly fed; mostly freshly hatched.
B 2 — Freshly fed; with traces of previous blood meal.
B 3 — Freshly fed; semi-gravid and still with old partially digested blood.
B 4 — Partial feeding; fresh blood.
C — Partially gravid.
D — Fully gravid with traces of old blood.
E — Fully gravid with old blood fully digested.
F — Miscellaneous; semi-digested old blood without ovarian development.

Black — opaque semi-digested blood.
Cross hatched — fresh translucent blood.
Stippled — ovarian development.

cold weather, greatly enhancing thereby the chances of malaria transmission during winter. This phenomenon occurs but rarely in India. However, Venkat Rao has also described another but related phenomenon of "gonotrophic discordance" which can be described as absence of any relationship between feeding and egg laying which can take place independently. Such phenomena of gonotrophic dissociation and discordance have been observed by many workers, but the degree and percentage of individuals in a population which show them very considerably and cannot precisely be stated. In Orissa, Venkat Rao (1943) estimated it to be about 20 per cent in *A. annularis* in rainy season.

The importance of gonotrophic dissociation has been recently discussed by Washino (1977). The phenomenon does not occur uniformly throughout the entire geographic range of a given species where it does occur. But the proportions of the blood engorged females that exhibit this phenomenon may sometimes be high. It may occur regularly in some areas but does not appear to be necessary as a survival mechanism in the majority, like other expressions of facultative diapause noticed in many insects. The gonotrophic discordance seems to be related to the short time of day light. The importance of these phenomena on malaria transmission needs to be studied more critically.

5. Hibernation and Aestivation

It is well known that in northern climates, such as in Europe and North America, many species of mosquitoes exhibit the phenomenon of hibernation. It may be described as the complete suppression of or minimisation of physical activity during very cold weather. Adults select a suitable shelter and remain quiescent without taking either blood meals or developing eggs. The mosquitoes draw their legs and wings close to the body and sometimes become frozen and stick to the surface on which they rest. But in several instances there may not be such a complete absence of activity and some feeding may take place similar to what occurs in gonotrophic dissociation. Such a hibernation has not been noticed with Indian anophelines, presumably because extreme cold temperatures are not found; but many females may overwinter in which case the gonotrophic cycle may simply become prolonged. The hibernating female usually develops excessive fat before it enters into the hibernating phase and utilises the fat during hibernation. The fat is gradually dissolved and if the adult does not appear by spring, it is probably dead.

Though hibernation of the kind and degree as found in the Palearctic and the Nearctic regions are not found in India, Chowdhury (1931) suspected hibernation in *A. subpictus* in Karnal (Haryana). While he found species like *A. annularis, A. culicifacies, A. stephensi, A. fluviatilis, A. nigerrimus* and *A. barbirostris* both as larvae and adults throughout the winter, he did not get *A. subpictus*. These observations, coupled with certain laboratory observations like the rapid death of adults and larvae in cold temperatures, led him to conclude that *A. subpictus* "would appear" to be a true hibernating species. Obviously, the evidence was not conclusive as it was not based on direct observation.

Rice and Mohan (1936), studying *A. minimus* in Assam had no difficulty in

collecting larvae and adults in nature throughout the cold season. They categorically showed that the species neither went into hibernation or overwintering nor was there any gonotrophic dissociation.

In the case of larvae of anophelines in India true hibernation has not been recorded in India though it occurs in some European species like *A. claviger* and certain species of *Aedes*. In India several species, particularly *A. minimus*, have been found to persist during the cold weather. The larvae continued to grow but at a very slow speed. This may not be strictly a case of overwintering or of diapause, which occurs in many species of insects, but only a slowing of the process of growth.

Aestivation is the act of remaining dormant during the hot season in contrast to hibernation. Animals, particularly of the deserts, remain motionless in ecological niches which provide shelter from the extreme dryness and heat of the atmosphere. There is no evidence to suggest that aestivation occurs in Indian anophelines. However, larvae of *A. moghulensis* are believed to be able to remain under moist sand for a long time till the breeding places again become flooded. This, however, is not true aestivation.

6. Larval Ecology

Growth: There are four stages in the growth and development of *Anopheles* larvae. The first stage larvae which hatch out from eggs are very small and are just visible to the naked eye. After imbibing the requisite food, the larvae grow and by a series of moultings, the second, third and fourth stage larvae emerge. Usually the moulted skin is eaten up by the emerging larvae except the moult of the fourth stage which is followed by the non-feeding pupal stage. It is difficult to identify the species of the first and the second stage larvae and most of the studies on morphology, chaetotaxy and behaviour have been made on the third and fourth stage larvae, particularly the latter. The size gradually increases at each stage from hardly 3/4 to 1 mm of the first stage of larvae, to the full grown larvae with lengths between 3 to 6 mm. The size, however, varies considerably between species to species, *A. hyrcanus* and *A. barbirostris* groups as also species like *A. gigas* have larvae of the largest size. The larvae of *A. aitkenii* group are among the smallest.

The time taken for the completion of the larval stages varies considerably, depending not only upon seasons and availability of food, but also on the species themselves. Normally, in the warm weather the length of the larval life ranges between 8 to 10 days. In cold weather, the duration may extend quite considerably; and some larvae in the colder parts of the country, such as northern and north-eastern India, may not pupate till March, i.e., they may live for 2-3 months. This is a mechanism evolved by insects to tide over the adverse conditions in cold weather. It is not hibernation, as life activities are not stopped, but only slowed down. The term 'overwintering' has often, but not quite accurately, been used for this process.

Breeding places: *Anopheles* larvae occur in many kinds of places. Any type of natural fresh water collection, unless highly contaminated by sewage or chemicals like factory effluents, can support the life of one or another species of *Anopheles*.

Larvae of *A. subpictus* have been found in Indonesia even to breed in hot sulphur springs. Though no species of *Anopheles* larvae are found in undiluted sea water, there are many species, such as *A. sundaicus*, which breed in highly brackish waters in lakes and creeks connected with the sea. Even *A. stephensi*, normally breeding in fresh water wells, is known to breed sometimes in highly saline waters such as sea water stored in barrels and diluted by rain. An interesting but extreme instance is the breeding of *A. sundaicus* in waters made saline by animal urine (Colless, 1948) in Borneo. At the other extreme are a few species like *A. umbrosus* and *A. balabacensis* which thrive in water in peaty soil found in monsoon forests; so also species like *A. aitkenii* which are found in deeply shaded arecanut garden trenches with a lot of rotting vegetation. These are, however, rather unusual situations.

Rain water, water from melting snow and irrigation water provide the sources of water for most types of breeding places. Underground water and water of springs are also dependent on rainfall or irrigation.

There have been many classifications of breeding places by almost every entomologist. The *Anopheles* breeding places in India are mainly of the following types:

Natural: Lakes, swamps, river margins, riverbed pools, streams, seepages, springs, saline marshes and tidal creeks, ground pools both in the open land and forests, footprints of animals such as elephants, cattle, etc., tree holes, coconut shells, bamboos, axils of leaves, crab-holes, oasis in deserts, etc.

Man made (*a*) Semi-natural: Large reservoirs for irrigation or for water supply, tanks, ponds, canals, ditches, channels and irrigation drains, rice fields, sugarcane and other agricultural fields with standing or flowing water, tea gardens and arecanut garden trenches, pits in casuarina and coconut plantations, quarry pits, borrow pits, brick pits, flooded cart tracks, clogged up drains, domestic and irrigation wells, etc.

(*b*) Artificial: Metal, plastic, glass, ceramic or earthen containers, metal or cement domestic water tanks, fountains, roof gutters in factories, barrels, buckets, pots and other vessels, antguards, broken bottles, discarded motor tyres, water used for curing cement in buildings, decorative projections above windows and doors which hold rain water, roof gutters with clogged outlets, water collections near leaky taps, containers made of wood, rubber, paper, etc.

Some species show wide diversity in the choice of breeding places while others are found in a few special places. *A. culiciformis* and *A. sintoni* are exclusively found breeding in tree-holes. Many of the species, however, are found in more than one type of breeding place. With special reference to Indian anophelines, some of the breeding places and the species *typically* found in them can be summarised as follows:

Tree-holes	*culiciformis, sintoni, barianensis.*
Marshes in jungles, animal footprints with rotting leaves and peaty water, slow running streams in shaded jungles	*umbrosus, balabacensis, aitkenii, insulaeflorum, bengalensis, barianensis, lindesayi, interruptus.*
Jungle and mountain streams	*barianensis, nilgiricus, gigas* and its varieties, *moghulensis, splendidus.* Sometimes *fluviatilis* and *minimus.*

Streams, irrigation channels, river margins, etc., with flowing water, springs and seepages	*culicifacies, fluviatilis, minimus, maculatus, barbirostris, majidi, kochi, theobaldi, stephensi* var. *mysorensis, subpictus, vagus, turkhudi.*
River and stream bed pools	*culicifacies, vagus, subpictus.*
Rain or irrigation water filled borrowpits, ground pools, etc., stagnant water without much vegetation except grasses	*culicifacies, subpictus, vagus, jeyporiensis, kochi, nigerrimus, barbirostris, tessellatus, stephensi* var. *mysorensis, pallidus, splendidus, jamesii, ramsayi, philippinensis.*
Pits in casuarina and coconut plantations	*culicifacies, subpictus* and *varuna,*
Tanks, ponds, borrowpits, swamps with stagnant water and abundant vegetation	*annularis, pallidus, philippinensis, jamesii, ramsayi, pulcherrimus, nigerrimus, jeyporiensis, aconitus, karwari, subpictus, vagus* (sometimes *culicifacies*)
Trenches, channels in arecanut, tea or sugarcane garden, etc. deeply shaded	*aitkenii, insulaeflorum, elegans, barbirostris, minimus.*
Rice fields, fallow or just planted	*subpictus, vagus, culicifacies, pallidus.*
Rice fields, growing	*nigerrimus, philippinensis, pallidus* (sometimes *fluviatilis, minimus* and uncommonly *culicifacies*)
Domestic wells	*stephensi* (type), *varuna, majidi, culicifacies.*
Field, unlined wells	*culicifacies, stephensi* (type) and var. *mysorensis (fluviatilis* during adverse seasons).
Brackish water swamps and lakes, coastal saltwater	*sundaicus, vagus, subpictus, annularis.*
Desert waters, pools in sandy soil	*multicolor, culicifacies, subpictus.*
Artificial containers, cisterns, fountains, kerezes, etc.	*stephensi* (type), *kochi, subpictus.*

While the above summary generally holds good for most places in India there are several variations and the selection of any breeding places by a species is not absolute because many of them breed sometimes in atypical breeding places particularly in adverse seasons. For example, *A. fluviatilis* normally preferring to breed in streams and channels and rice fields may occasionally be found in wells or tanks and ponds. *A. nigerrimus* normally found in rice fields may also be found sometimes in channels and swamps. *A. sundaicus* normally preferring saline water has been found to breed in fresh water. *A. minimus* is sometimes found in ricefields, wells and borrow pits. Such variation in distribution may be the result of unusual factors forcing the gravid females to deposit eggs in atypical breeding places or the larvae might be carried to such places by water currents. However, even recognising that there is no such thing as an absolutely fixed behaviour in nature, there are certainly preferred types of water for most species. Some species may be catholic and some may be very selective. The details are given under the respective species.

Temperature: The temperature of water in breeding places plays an important part in the persistence and growth of larvae. First, larvae cannot survive extremely high temperatures. The upper limit of temperature at which no larvae can survive is

known as the 'thermal death point'. Secondly, the speed of growth is accelerated in warm waters and lessened in cold water. Thirdly, higher temperatures stimulate growths of aquatic plankton and provide more food to the larvae than do cold waters.

The work of Muirhead Thomson showed different thermal death points for different species; for example, *A. minimus* 41.0°C, *A. vagus* 44.5°C, *A. culicifacies* 44.0°C, *A. nigerrimus* 43.0°C to 43.5°C. While species like *A. vagus* and *A. subpictus* could survive even in shallow waters of rice fields and ground pools exposed to the direct sun and with temperatures upto 44.0°C, *A. minimus* larvae could not withstand temperature of over 40.0°C for more than a few minutes.

It is, however, not clear whether temperatures of natural breeding places influence the gravid females to exercise a choice when egg laying. In the laboratory some species did exhibit a choice, as shown by Pal with *A. culicifacies* and *A. fluviatilis*, but Muirhead Thomson and the present author have found that the temperature of most breeding places is, more or less, very similar in the early part of the night whatever might have been the temperature during daytime or late at night. As most species of anophelines seem to lay eggs to a greater extent in the early part of the night, though egg laying may continue throughout the night, the temperature of water was not likely to be the determining factor because of the similarity of temperatures of the breeding places at that time. However, gravid females may be able to perceive even very slight differences in temperature.

At 52.0°C all larvae die immediately. This is the limit of biological tolerance of temperature. However, such temperatures are not found in the breeding places in nature in India.

Though very precise information is not available in respect of Indian species, freezing kills anopheline larvae. There is an extensive literature on this subject with European species. Many species can survive in cold waters if they are not actually frozen, even if the water is supercooled. But the moment they come in direct contact with ice they die.

Shade: Many species of anophelines, such as *A. culicifacies, A. subpictus, A. vagus*, etc., breed and survive both in open waters with very little or no shade, as in borrowpits or river bed pools and also in places with some shade. However, species like *A. minimus* and *A. fluviatilis* are found usually in slightly shaded breeding places either under shade from overhanging trees and bushes or from thick growths of grass. Deep shade is, however, not suitable to the larvae of *A. minimus*. The work of Muirhead Thomson has shown that *A. minimus* adults actually showed a preference to deposit eggs in shaded places. But he also found that deep shade resulted in the disappearance of grass along the margins of streams and channels, thereby increasing the velocity of water and, paradoxically, having an adverse effect on larvae. He found that streams and channels without overhanging dense shade were quite suitable if there was good growth of grass which itself provided a certain degree of shade.

A. fluviatilis occurs in shaded breeding places provided the flow of water is slight. The present author has collected *A. fluviatilis* in streams with a slow flow of water

under overhanging shade and without any grass. Many species such as *A. umbrosus, A. balabacensis, A. aitkenii*, etc., breed only in dense shade of forest or plantations. The Sun loving *A. maculatus* disappears when the breeding places are shaded. But the exact mechanism of it is not understood. Shade might also have an indirect adverse effect on the growth of plankton including algae which may adversely influence breeding. Species which breed in deep wells, such as *A. varuna* and *A. stephensi*, remain in shade for most part of the day. In fact, *A. stephensi* grows well in cisterns or covered wells which never get any direct sunlight.

The preference shown by *A. fluviatilis* larvae in the laboratory for slight shade and the probable mechanism of negative phototaxy are mentioned in the section related to that species. Several shade-loving species, such as *A. balabacensis* and *A. umbrosus*, actually breed in still waters of forest pools. In their cases there is no question of the larvae being flushed away by currents, though some overflow of the pools during heavy rains is probable.

Movement of water: Slow flow of water is a requisite for several species, particularly *A. fluviatilis* and *A. minimus*. These two important vectors of malaria in the country are generally found in streams, channels and other breeding places such as rice fields in which there is a perceptible current. But generally the larvae of these species do not continue to occur in places where clean weeding has been done. In fact clean weeding has been utilised as an effective means of control of *A. fluviatilis* and *A. minimus*. Strong currents without providing opportunities for clinging to suitable supports are not favourable for the larvae.

Muirhead Thomson (1939-42) has shown that even in channels the actual location of the larvae of *A. minimus* is inside pockets or along edges where the flow is negligible. The question which arises is whether the flow of water *per se* is attractive or some other factor. The present author has found *A. fluviatilis* in certain situations where there is no recognisable flow, but where there was a constant replacement of water, as along shallow edges of jungle streams with hardly one or two mm depth of water. In such places there was constant replacement of water which perhaps itself provided a degree of current which the larvae could perceive.

Muirhead Thomson showed that ripples of water did not affect egg laying by *A. minimus*. He also showed that under laboratory conditions, the species showed a definite preference for oviposition in still water than in running water.

Iyengar (1922) had shown that species like *A. minimus* and *A. maculatus* were able to stay in running water because of their ability to cling to surfaces at the edge by means of the tail hooks. Muirhead Thomson showed this to be incorrect. The presence of larvae of *A. minimus* in grassy margins was due to a strong avoidance of light which directed them to clumps of grass, etc., which provided shade and minimum of water movement.

There are many species which are indifferent to flow of water. Species like *A. culicifacies* and *A. subpictus* breed as readily in stagnant as in flowing water. However, few species, if any, can withstand strong flushing effects.

A. annularis, A. jamesii, A. pallidus, A. nigerrimus and several other species are

predominantly breeders in still water. The inability of larvae of many species to withstand strong currents has been used as a means of control by several types of flushing devices such as siphons, hand operated sluices, etc.

Other physical factors: Anopheline larvae rest below the surface of water by clinging to the surface with their hairs along the thorax and abdomen and by the spiracular plates. Often it has been thought that if the surface film is not strong enough to provide support, the larvae may be unable to cling and therefore unable to survive. Russell and Ramachandra Rao (1941b) found in laboratory experiments that if the surface tension of water, which is normally 72 dynes per centimetre was reduced to about 28-30 dynes by gradual addition of soapy water *A. culicifacies* larvae were unable to cling to the surface. If the larvae were retransferred immediately to fresh water they regained that ability. However, when surface tensions of waters of many common types of natural breeding places were measured, such a low surface tension of water was not found. Surface tensions of natural breeding places did not seem to have any significance in the distribution of *A. culicifacies* larvae. In a similar study made by Renn (1914) in U.S.A., the surface tension in various natural breeding places in which *A. quadrimaculatus* was breeding varied between 65 to 73 dynes per centimetre.

Chemical contents of water: The pH of natural waters varies considerably. In the present author's experience in South India, the pH ranged anywhere from 6.0 or even less in deep wells to 11.0 in tanks and ponds. The acidity in the wells was mainly due to dissolved CO_2, and once it was removed the residual pH was nearly 7.8. Wells were the preferred places of *A. stephensi* and *A. varuna*. But it was not clear whether it was the acidity which made wells the favourable places. The open waters in tanks and ponds harboured species like *A. annularis* and *A. jamesii*. It was more probably due to the presence of vegetation rather than pH.

In Bengal, Sen (1938b) found the pH to range from 7.5 to 8.5 with an average of 8.2 in breeding places of *A. sundaicus*. It was definitely alkaliphilic. But *A. sundaicus*, like *A. annularis*, also likes growths of vegetation. It was again not clear whether it was pH *per se* or vegetation, which made certain breeding places favourable.

Silt: The presence of silt has often been believed to have an inimical effect on some species of *Anopheles* larvae. Both with *A. culicifacies* and *A. minimus*, it is now known, silt is not such an adverse factor. Similar conclusions have also been drawn with regard to *A. philippinensis*. Species like *A. subpictus* and *A. vagus* are well known to thrive well in silt laden water.

Salinity: Most Indian anophelines breed in fresh non-saline waters. However, a few species like *A. stephensi, A. subpictus* and *A. annularis* can tolerate high degrees of sodium chloride salinity. *A. stephensi* has been found by Challam (1924) to breed in sea water stored in barrels. *A. stephensi* and *A. varuna* breed in deep wells, some of which have very hard waters, but the salinity may not be due to sodium chloride but due to other salts. *A. annularis* has been found to breed even in the Chilka lake in Orissa along with *A. sundaicus*. *A. subpictus*, the most ubiquitous *Anopheles* in India, can breed in salt marshes. The present author has

collected this species in the coastal salt marshes in Adiramapatnam, near Pattukottai in South India. Even differences between salt water and fresh water forms of *A. subpictus* have been recorded (Sunderasan and Appa Rao, 1943).

A. sundaicus is *par excellence* the salt water breeder. It has been found extensively in Salt Lake area in Calcutta, in Orissa coast (Chilka Lake) and in Andhra Pradesh coasts. Salinities from 0.15 to 2.3 per cent have been recorded; but the optimum salinity range, according to Senior White and Adhikari (1939) was 0.87 per cent, and according to Iyengar (1931) 1.5 to 2.0 per cent (see details under *A. sundaicus*).

Flora and fauna of the larval environment: The relationship between anopheline breeding and the presence or absence of vegetation and aquatic fauna has received much attention. It is well known that certain species can breed in natural waters in which there is no visible flora and fauna, and other only in the presence of various kinds and degrees of growth of vegetation. It would not be correct to say that in the former case there would be no flora and fauna at all because natural waters usually contain many types of unicellular organisms, particularly diatoms, in the plankton without which anopheline larvae would not be able to obtain the food required for their growth. Anopheline larvae cannot grow in pure distilled water, but have been grown in waters containing proper combinations of certain inorganic salts.

Under laboratory conditions anopheline larvae can grow vigorously in containers to the water of which have been added various types of food additives. Hay or straw infusions containing rich growths of protozoans, such as flagellates and ciliates, have been used routinely in many laboratories. Yeast powder, dog-biscuits, litmus milk, etc., have been commonly used. Larvae of almost any species of anopheline can be grown in the laboratory with such food aids.

In nature the conditions are very different. Species like *A. subpictus, A. vagus* and *A. culicifacies* often grow in temporary pools of water without any visible growth of vegetation. In certain situations, however, as in very long-standing breeding places as in river bed pools or borrowpits, they thrive equally well in the presence of filamentous green algae such as *Spirogyra*. Some of these pools or borrowpits have many planktonic organisms including green algae, such as Chlorella, Chlamydomonas, Volvox, Euglena, etc. But when members of blue green algae appear, *A.culicifacies* begins to disappear. Even temporary breeding places contain many species of diatoms on which alone larvae can thrive. Dissection of larval guts has shown an abundance of diatoms and unicellular algae.

At the other extreme are species, like *A. annularis, A. jamesii, A. pallidus, A. sundaicus, A. philippinensis, A. nigerrimus* and others, which thrive only in waters rich in vegetation. Vegetation of such waters includes:

(A) *Planktonic* forms such as unicellular algae, protozoans, diatoms and bacteria.

(B) *Lower vegetation* particularly multicellular algae including filamentous green and blue green algae, some of the latter forming thick mucilagenous floating masses, such as *Lyngbia*.

(C) *Higher vegetation* including:

(i) floating vegetation, such as *Nelumbium, Eichhornia, Pistia, Lemna, Wolffia,* etc.
(ii) emergent vegetation: various types of grasses, bull rushes, etc., and
(iii) submerged vegetation: *Vallesneria, Ceratophyllum, Elodea, Potomogeton, Hydrilla,* etc. The tips of many of the submerged vegetation actually reach the surface and provide shelter.

While moderate growths of vegetation can provide optimum conditions for growth of larvae, abundance of some of the floating forms actually lessens the intensity of breeding, as in the case of water hyacinth and *Lemna* with regard to *A. philippinensis.* Thick growths of vegetation, except grass, tend to inhibit the breeding of species like *A. culicifacies, A. fluviatilis, A. minimus, A. vagus* and even *A. philippinensis.* On the other hand, thick masses of uprooted and decomposing vegetation on the surface favour the growth of *A. sundaicus.* A uniformly complete coverage of water is not favourable; but if the vegetation is dispersed with many open places, it is favourable to larvae.

Vegetation has the effect of increasing the intersection line, i.e., the length of the water edge. It has been shown in studies in U.S.A., such as those conducted in the Tennessee Valley, that the increase of the intersection line increases the shelter for the larvae and leads to increase in their numbers. Such studies have not been made in India. Reference, however, can be made to the work of Kachroo and colleagues who attempted to understand the factors leading to mosquito breeding in large lakes as in the Damodar Valley Project.

Wells, as a general rule, do not have rich growths of vegetation except a few growths of floating algae. But along the surface of walls of the wells submerged under water slimy growths of certain species of algae can be seen. The water itself would be clear, but will contain many planktonic organisms. Species like *A. varuna* and *A. stephensi* seem to prefer wells as their breeding places. But the exact reason why they prefer wells—whether because of the type of vegetation or the shaded cool and clear water—are not exactly known. Perhaps it is the physical character of the well which is attractive to the larvae.

Mountain streams are also generally free of rich growths of higher vegetation. But the rocks and pebbles in flowing water are generally covered by slimy masses of algae. Species which breed in such places include *A. minimus, A. fluviatilis, A.g. simlensis,* etc.

The association of *A. nigerrimus* with growing rice fields has been noted all over India. What makes this habitat particularly attractive to this species, whether the rice plants themselves or other related vegetation, is not known. The mechanical obstruction provided by rice plants which inhibit *A. culicifacies* breeding is dealt with under that species.

No direct evidence of the actual inimical effect of any plant on any species of Indian anopheles has been obtained except that *Cyanophyceae* (blue green algae) are definitely unfavourable to *A. culicifacies.* The green alga, *Euglena viridis,* is usually not unfavourable. But the author has found that in small ponds with rich growths of *Euglena haematoides,* larvae of all species are scarce. This *Euglena* has

plastids of dark red-brown colour which give the ponds during mid-day a dark brown appearance. Among filamentous algae *Anthophysa* sp., which grow in highly ferruginous waters, was unfavourable.

Covell (1941b), in his book on anti-malaria measures, has grouped inimical aquatic vegetation into three types:

(i) Thick growths on the surface actually preventing breeding, e.g., *Lemna, Azolla, Wolffia, Anacharis, Trapa,* etc.

(ii) Those which act as traps, e.g., *Utricularia* i.e., bladderworts, which are known to entrap and digest insects including mosquito larvae.

(iii) Those which are actually poisonous, e.g., *Chara.*

There are many references to the action of *Chara*, both as regards its inimical nature and harmlessness in other countries. The present author collected many tank-breeding species such as *A. annularis, A. jamesii,* etc., in the presence of good growths of *Chara* southern India and found that they had no effect. If there is any specificity of effect against particular species, it is not known.

In short, vegetation of breeding places does have a profound influence on the distribution of anopheline species. However, while gross effects have been studied, the exact mechanisms by means of which anopheline species are influenced have not been studied adequately.

Regarding aquatic fauna, it should be noted that apart from providing food, as in the case of protozoans, flagellates and ciliates, some of them are inimical to anopheline larvae. When there are heavy growths of *Vorticella* they smother the larvae. The predatory effect of certain insects and higher forms of animals, such as fish, are also well known. They will be dealt with in the section on parasites and predators.

Important work has been done on the relationship of vegetation and anopheline larvae by workers like Sen, Senior White, Kachroo and others which are detailed under respective species. An example of a study in Bengal is described below:

The role of aquatic vegetation in its relation to anopheles breeding has been well studied particularly in the deltaic areas of Bengal. An exhaustive study was made by Sen (1941c) and it would be best to give a summary of his findings in his own words.

1. Anopheline breeding and emergence in relation to the presence of various aquatic plants, *Spirogyra* sp. *Ceratophyllum demersum, Hydrilla verticillata, utricularia flexuosa, Ipomoea reptans, Pistia stratiotes, Lemna minor, Limnanthemum cristatum, Najas foveolata, Ottelia alismoides, Hymenachne myurus* and *Echhornia* spp. have been studied in certain villages in Lower Bengal where malaria is endemic.

2. Altogether 12 species of anophelines, *A. hyrcanus* var. *nigerrimus, A. barbirostris, A. ramsayi, A. annularis, A. philippinensis, A. pallidus, A. jamesii, A. subpictus, A. vagus, A. varuna, A. aconitus* and *A. culicifacies,* bred and emerged from waters containing the plants mentioned above.

3. The presence of *Pistia stratiotes, Ceratophyllum demersum* and *Spirogyra* sp. was compatible with heavy breeding of the several anophelines recorded. The proportion of adult emergences from tanks containing these plants was also high.

4. The emergence rate was very low in association with *Utricularia flexuosa, Ottelia alismoides, Najas' foveolata* and *Hymenachne myurus*, although the numbers of anopheline larvae found in such breeding places were by no means insignificant.

5. *Lemna minor*, when growing thickly, appeared to be detrimental to anopheline breeding; *A. subpictus* and *A. vagus* were the chief species associated with this type of vegetation.

6. *A. ramsayi* was almost exclusively associated with *Pistia stratiotes. A. nigerrimus* also appeared to have a strong preference for breeding places containing the plant, while this species seemed to avoid *Ceratophyllum* and *Hydrilla* where other vegetation is present.

7. Waters containing *Hydrilla verticillata* and next to this *Ceratophyllum demersum* were most frequently associated with larvae of *A. annularis*.

8. Larvae of *A. philippinensis* were recorded from water containing all the types of plants studied excepting *Lemna*; but the species was most frequently associated with *Spirogyra* sp. *A. subpictus* was also frequently associated with this type of vegetation.

9. Larvae of *A. pallidus* were most frequently found in association with *Utricularia*. The virtual absence of emergence of the species in the area may be due to the destructive action of the plant on the aquatic stages of species.

10. For waters containing water hyacinth, *Eichhornia speciosa, A. aconitus* showed a special predilection.

11. The proportion of emergence of the different anopheline species, as well as the number of larvae, varied according to the types of aquatic vegetation present and the season of the year when the observations were made.

12. With regard to the number of different anopheline species associated with each type of plant, *Pistia* stands first, closely followed by *Eichhornia, Spirogyra. Limnanthemum, Ipomoea* and *Najas*. But as regards larval density *Hydrilla* and *Najas* stand at the top of the list.

A final point which needs to be made in regard to larval ecology is that it is desirable to make careful studies, experimentally if possible, to determine the exact factors responsible for the breeding or non-breeding of a species. Some of the factors like shade or vegetation may not themselves be responsible, but they may indirectly influence some other factor which may be the stimulating factor. For example, it is not the rice field water but mechanical obstruction of vegetation which prevents the breeding of *A. culicifacies*. It is not shade but the destruction of vegetation which increases water velocity and drives out *A. minimus*. It is not water hyacinth by itself, but its effect on plankton which influences breeding of *A. philippinensis*. Unless critical studies are made, one may draw erroneous conclusions.

7. Biology of Eggs

The eggs of *Anopheles* are laid singly and actually fertilized as they pass through the atrium by the spermatozoa stored in the spermatheca. The develop-

ment of the larva takes place inside the egg but there is no indication that the egg draws any nourishment from the environment. A slight degree of enlargement of the egg has been noticed, perhaps due to absorption of moisture. The time taken for the complete development of the egg varies to some extent. In normal circumstances in the Indian climate, it takes about 24-48 hours, it may become prolonged under colder conditions.

The eggs cannot withstand long desiccation, unlike some species of *Aedes* the eggs of which remain viable even months after the habitat becomes dry. The eggs of *Anopheles* cannot also be stored on dry filter paper as eggs of certain *Aedes* spp. can be. For the survival of the eggs and the development of the larvae, even a thin film of water is sufficient. Often it has been noticed that first stage larvae emerge out of the eggs when maintained on wet filter paper.

The eggs float on the water surface and, unlike larvae and pupae, cannot withstand long submergence. Bhatia and Wattal (1958) found that eggs of anophelines submerged for long periods (beyond 92 hours) failed to hatch out. They worked with eggs of *A. annularis, A. culicifacies, A. stephensi* and *A. subpictus*. The hatching viability decreased as the number of hours of submergence increased.

Pal (1945a) studied the viability of eggs of *A. culicifacies* under various conditions of temperature. He found that the most favourable range was 28°C to 36°C. Both lower and higher temperatures, 20°C and 40°C were unfavourable. The thermal death point for eggs was 53°C though even at 50°C only 12 per cent of eggs hatched. (It is, however, to be noted that 52°C is absolute lethal temperature for most living organisms without internal temperature regulatory mechanisms). At that temperature protoplasm is destroyed. It was not unexpected that all eggs died at this temperature. In fact when larvae have to be killed suddenly for purposes of mounting, they are dropped into water of 52°C.

5°C was the lowest temperature tolerated by eggs. Pal stated that as the lowest temperature reached in natural waters in the Punjab was about 8°C, uninterrupted breeding, although greatly slowed down, can occur during winter also.

Muirhead Thomson (1940c) studying *A. minimus* eggs experimentally, has recorded that the time taken for hatching varied with temperature:

35°C and 30°C .. 2 days 25°C .. 2.5 days 20°C 3.5 days and 16°C .. days.

It has been found with *A. gambiae*, during its invasion of Brazil, that when eggs were stored in contact with moist sand, the maximum period of survival was 19 days, but the rate of survival rapidly decreased as the number of days increased.

The number of eggs laid by an individual female varies considerably. It usually ranges between 100 and 150. The quantity of blood ingested has perhaps some influence on the number. Seasonally there did not appear any difference in South India in *Anopheles culicifacies* (see under *A. culicifacies*). It varied from 105 to 135 in a total of 163 females.

It is well known that often abnormal eggs are found, including those which are infertile. The reasons are not understood. But it could be due to mating with incompatible individuals or it could be due to exposure of the gravid female to low

temperatures. Eggs are not known to be susceptible to insecticides such as DDT or HCH.

8. Biology of Pupae

The pupal stage is one of the four stages of *Anopheles*, least studied from the biological point of view.

Pupae are free living with great motility unlike in many Diptera where they are immobile. The pupae are lighter than water and when it is not actively moving, it floats up to the water surface. The buoyancy is perhaps due to large air spaces in between the "wing cases" on the underside of cephalothorax. It does not, therefore, need hairs and hooks to cling to the surface of water, but it is in contact with the film with the aid of the two large trumpets (acting as "spiracles") and two long stellate hairs of the abdominal segment 1.

The large paddles and the flexion of the abdomen help the pupae to move actively in jerks. The pupae may when disturbed physically or by vibration, sink under water for a short time but soon float up to the surface.

Pal (1945) noted that under laboratory conditions *A. culicifacies* pupae hatched well at 28°C to 32°C; when the temperature was elevated to 36°C to 40°C only 30 to 40 per cent hatched out.

The *metamorphosis* is usually completed between 24 to 48 hours in the normal conditions in peninsular India. It is certainly prolonged in cooler climates. Muirhead Thomson (1940c), working with *A. minimus*, found that the length of the pupal stage varied with temperature:

at 30°C, it was 1.25 days, at 24.0°C .. 1.75 days, at 20°C .. 2.75 days, at 16°C .. 4.5 days.

There is some uncertainty whether the time of pupation or the emergence of the adults occurs at any particular time of the day, whether at night, daytime or during evening. Sen (1935) found with eight common species of Bengal that they all pupated at daytime. Other authors in Southeast Asia also have found pupation to occur during daytime.

9. Parasites and Predators

It is very well known that natural populations of mosquitoes are kept under check by the activities of parasites and predators. Much work has been done on this aspect in India and in many other countries. A major review of parasites and predators would be beyond the scope of this book. However, it would not be complete without reference to some of the major findings particularly in India.

Larvivorous fish, both introduced and indigenous, have been recognised as the most effective of all natural predators in the country. In addition, the following groups of animals and plants are known to play some part:

Vertebrates: Bats—It is a common observation at dusk that large numbers of insectivorous bats are found flying and seen devouring many flying insects, including mosquitoes.

Birds — There is not much evidence of birds having a role in this regard. However, there are many insectivorous birds, some of which may devour mosquitoes in the evenings.

Reptilia — Many types of lizards, particularly the house gecko, consume mosquitoes in houses and cattlesheds. The chameleon is known to pick up insects by extending its long tongue.

Amphibia — There are definite records of frogs and toads eating insects, including mosquito larvae in the breeding places.

Fish: Several species of fish, such as *Gambusia affinis, Aplochilus panchax, Poecelia holbrooki,* etc., are very effective predators of anopheline larvae. A useful list of indigenous fish which eat mosquito larvae has been given by Prasad and Hora (1936).

Among invertebrates many predators and parasites can be mentioned.

Arthropods: *Arachnida* — Web building spiders trap and devour many adult mosquitoes inside houses and cattle sheds.

Arcarina — Certain members of the mite family, *Hydrachnidae,* are found to infest larvae of anophelines, sometimes in such large numbers that the larvae die. Sometimes the mites are found on adults also, presumably infested at the time of eclosion.

Insects — There are many insect predators of mosquito larvae. The larvae of dragon-flies (Neuroptera), water scorpions, many types of water bugs, such as *Nutonectidae, Corixidae* and others, destroy quite a large number of mosquito larvae. Adults of dragon-flies particularly in the evenings, devour many anopheline adults which are also on the wing at that time. The author has noticed that, even during daytime, dragon-flies are attracted to mosquitoes. It has been observed that anophelines irritated by pyrethrum sprays attempt to escape from the eaves of thatched houses when spraying is in progress and swarms of dragon-flies follow the spraying squads and devour large numbers of the escaping adult mosquitoes. On dissection, the captured dragon-flies have been found to have in their stomachs the remnants of a large number of mosquitoes (Personal observation of the author).

Even many species of mosquitoes, such as *Toxorhynchites,* are known to eat mosquito larvae found in their breeding places. The cannibalism exhibited by some of these mosquito larvae is so great that sometimes only one larva of the species persists in a tree hole. *Toxorhynchites* sp. have been used in experimental control of certain species of *Aedes* mosquitoes. The predatory habits of *Culex* (*lutzia*) sp. are well known.

An interesting phenomenon noticed by many entomologists in the habit of the adults of *Culicoides anophelis* actually attaching themselves to the abdomens of freshly fed anophelines, and taking their own blood meal by piercing the mosquito stomach, but its effect on the longevity of mosquitoes is not known.

The use of other insects, particularly *Anisops bouveri,* has been attempted in experimental control of *Anopheles* larvae. The insect has also been mass-reared for this purpose. It works very well in laboratory but results in the field have not been satisfactory (VCRC reports).

Nematoda: *Mermethids*—Several species of mermethids are known naturally to infect *Anopheles* larvae and sometimes even adults. The infected larvae invariably die. The present author has seen as many as four out of five larvae so infested, particularly in *A. annularis* collected from tanks. The use of mermethids for control of *Anopheles* larvae in natural breeding places has been seriously considered and experimented with. Apart from experiments in other countries, studies on *Romanomermis* have been carried out at the Vector Control Research Centre at Pondicherry. The mermethids have been mass reared and introduced into natural habitats. The results have so far not been encouraging in India.

Parasitic Protozoans: Parasitic protozoans play an important role in controlling natural populations of mosquitoes. The most important of them are species of *Nosema, Pleistophora, Stempellia* and *Thelohania.* In India only *Nosema* and *Thelohania* have been tested. Recently it has been shown that infections with *Nosema* may also lead to lessened susceptibility of *Anopheles* to plasmodial infections (VCRC report). Another protozoan of considerable interest is *Vorticella* found in natural waters; sometimes they attach themselves to the bodies of larvae of anophelines in such large numbers that the larvae are practically smothered. *Vorticella* grow very profusely in hay infusion used for rearing *Anopheles* larvae in the laboratory.

Parasitic Fungi: Mosquito larvae are also parasitized by the several species of fungi, which lead to high mortalities in natural populations. There are many species which are involved, but the one which has been extensively studied is *Coelomomyces.* Some of these are exclusively parasites of mosquitoes. They have been studied both in India and abroad.

Pathogenic Bacteria: There are several species of bacteria which infest mosquito larvae. *Bacillus thuringiensis* and *Bacillus sphaericus* have been extensively studied. These have been cultured and even used as "microbial insecticides" to deal with a variety of insect vectors of medical and agricultural importance. Studies are being now carried at the Vector Control Research Centre at Pondicherry. *B. sphaericus* has been isolated in India and is found to be quite effective, but safety tests are yet to be done.

Plant enemies: In addition to animal parasites and predators, reference can be made to the action of several plants which are directly or indirectly inimical to *anopheles* larvae. *Utricularia* spp., the bladder-wort, is known to reduce mosquito populations. It is a carnivorous plant found extensively in India. Reference has already been made to the alga, *Chara,* as inimical to larvae, but it is not well substantiated. Certain seeds of the members of the mustard family of plants form a mucilagenous product which entrap mosquito larvae.

The brief and general account of parasites and predators is given only to draw attention to their importance in mosquito ecology. There are many published documents which are of interest and have been dealt with extensively in many text books. A very interesting pamphlet *Mosquito Control — Some Perspectives for Developing Countries,* prepared by National Academy of Sciences, U.S.A. in 1973.

Most of the parasites and predators mentioned above occur in India and have

been referred to in literature. Given below is a brief list of some of them recognised naturally in India, (Courtesy: Dr. P.K. Rajagopalan), except fishes and higher animals.

PROTOZOA

Thelohania sp.	*A. subpictus* larvae	V.C.R.C. records
Nosema algerae	*A. stephensi* larvae	Geetha Bai *et al.* (1979)

BACTERIA

Bacillus sphaericus	*Anopheles* spp. larvae	Singer (1975)
B. thuringiensis	from soil?	Balaraman. (1983)
	"	V.C.R.C. reports

FUNGI

Coelomomyces anophelesicus	*A. annularis* larvae	Iyengar (1935, 1962)
	A. subpictus larvae	"
	A. vagus larvae	"
	A. varuna larvae	"
C. indicus	*A. aconitus* larvae	"
	A. annularis larvae	"
	A. barbirostris larvae	"
	A. jamesii larvae	"
	A. nigerrimus larvae	"
	A. ramsayi larvae	"
	A. subpictus larvae	"
	A. varuna larvae	Chandrahas and Rajagopalan (1979)
Aspergillus parasiticus	*A. subpictus* larvae	Hati and Ghosh, (1965)
Aspergillus sp.	*Anopheles* larvae	Christophers (1932)

INSECTS

Culicoides anophelis	Many species of *Anopheles* adults	Several authors
Dragon-flies adults	" "	"
Dragon-flies larvae	*Anopheles* larvae	"
Anisops sp.	*Anopheles* sp. larvae	Panicker and Rajagopalan (1977).

MITES

Water mites	*Anopheles* larvae and adults	Several authors

HELMINTHS

Romanomermis	*Anopheles* sp. larvae	Gajanana *et al.* (1978)
Mermethidae Undetermined sp.	*Anopheles* sp. larvae	Many observers.

10. Anophelines as Cause of Nuisance

In common with all blood feeding mosquitoes, anophelines sometimes cause irritation and itching of the part of the skin bitten. However, they are rarely cause for severe nuisance in the manner that *Culex* and *Aedes* species are. Certain species, such as *A. vagus, A. subpictus, A. nigerrimus,* etc., do cause some annoyance because of large numbers. The well known cliche that *Anopheles* females neither "sting nor sing", and therefore do their work unnoticed in contrast to Culicines, is largely true.

5

Speciation, Genetics and Cytogenetics of Anophelines

The science of genetics has been very useful to mosquito entomologists, enabling them to understand and unravel many aspects of mosquito taxonomy and biology. Though the advances in the field have been the greatest in respect of culicine mosquitoes, particularly of the members of the genera *Culex* and *Aedes*, some progress has been made in regard to anophelines also. Many examples of the application of principles of genetics to mosquitoes can be found in such comprehensive works as those by Mattingly (1976), Wright and Pal (1967), Davidson and Mason (1963), Colluzi and Kitzmiller (1975), Kitzmiller (1976), Pal *et al.* (1981) and others. Among the aspects which have received attention are speciation, hybridization, formal genetics, inheritance of susceptibility to pathogens and to insecticides, and relationship to behaviour. Attempts have also been made to use genetic information and techniques for the control of natural mosquito populations.

Many studies have been made on cytotaxonomy. Some of the significant developments as they relate to Indian anophelines are summarized in this section.

Concept of Species and Subspecies: In the biological world, which is governed by constant evolutionary processes, there are many observable or inapparent variations in the same animal or plant. They often occur in a continuity of forms and the characters form a range of variations at the extremes of which two distinct forms may be recognised, whether in shape, size, colour, measurements of parts, differences in the characters of scales or hairs, and sometimes in behaviour. But are they really two different species or merely extreme forms of the same species? Such questions are difficult to answer. As someone has said "Species are merely islands in the sea of life". After two distinct forms have emerged, the intermediate forms often disappear, leaving the two extreme forms which become adopted to their environments and become genetically isolated.

In higher forms of life, including mosquitoes, which reproduce sexually, a species is a "Genetically distinctive, reproductively isolated natural population" (Encyclopedia Britanica, 1961). According to Mayr (1977), "Species are a group of inbreeding natural populations that are reproductively isolated from other such species." Genetic distinctiveness is the common denominator for all valid, qualitative and quantitative species characters, including the cytological, embryological, ethological and morphological. The test is that there should be interspecific sterility. Two forms which cannot mate or if they mate they cannot produce fertile offspring are to be regarded as distinct species. This holds good for most of the species of

animals, including mosquitoes. But sometimes the interspecific sterility is not so complete and fertile progeny do arise, though generally they are less than normally fertile.

Till recently taxonomy was confined mainly to distinguish and describe morphologically different forms. New species were named if the variations were clear and all or the majority of individuals in a population exhibited them. Most of the descriptive taxonomy was based on the study of killed and mounted specimens, sometimes on the basis of a single specimen. Genetic studies were rarely made. However, in spite of few glaring errors, the older methods have been able to identify a large number of distinct species on the basis of morphology alone.

On the other hand evidence is gradually mounting that "Cryptospecies" or "Sibling species" occur. Externally there may be no detectable morphological differences but populations may vary markedly in behaviour and relation to disease transmission. In the normal classical taxonomy they are placed under the same species, though they may, in fact, be two or more different species, easily detectable if mating experiments are conducted. In India, for example, there are strong grounds to believe that there are some cryptospecies or sibling species in *A. fluviatilis, A. culicifacies, A. philippinensis, A. subpictus,* etc., apart from the now well known forms of *A. stephensi.*

Sibling species are those which are reproductively fully isolated and ecologically distinct but with barely recognisable, if any, morphological differences. The *"maculipennis"* group in Europe is an excellent example. Marked differences in ecology and behaviour and relationship to disease had been noticed in the field long before they could be recognised morphologically by the study of the patterns on the surface of eggs (Hackett *et al.*, 1932). More recently cytological studies have shown differences in chromosome morphology also (Kitzmiller, 1976). Members of the *"gambiae"* complex in Africa are other examples of sibling species.

Polymorphic species is a term used to denote a valid single species in which more than one form with morphological differences occur. They mate readily and produce offspring in which both types may be found. A notable example is *Aedes aegypti* in which three distinct geographical forms are known with morphological differences; but all inter-breed readily. Each of the three forms, namely the type form, *formosus* and *queenslandiensis,* is adapted to its own environment and zoogeographical region. So also are several forms of *Culex pipiens.* Among Indian anophelines the example of *A. maculatus* may be cited. Reid *et al.* (1966) found two forms in the Himalayas distinguishable by slight morphological differences, but no recognisable differences in habits (see also Senior White's notes described later). There are many examples of variations in external morphology, usually used for identification which may be examples of polymorphism. Wattal *et al.* (1960) have described numerous types of variations in Indian anophelines which can be the result of polymorphism. The variety of *A. subpictus,* var. *vadakadiensis,* described by Doraisamy (1963) is perhaps a mere polymorphic form. One should be very careful in naming species or subspecies on the basis of trivial variations unless some basic hybridization experiments are done or the form is reared to a few generations

to see if the character breeds true. Of course, with many species direct crossing is not feasible. Both the above types of forms, namely sibling species and polymorphic species come under *sympatric complexes.* Sympatric forms are those which live in the same geographical area and often in the same habitats.

On the other hand, forms which occupy different geographical areas or occur in the same area but in quite different seasons are known as *allopatric forms.* They are characterized by small variations in colour, size or chaetotaxy. Though they are not genetically isolated, they have developed the variation because of ecological differences, for example, *A. subpictus* of India and *A. subpictus* of Indonesia. Such allopatric forms go by the name of varieties which because of certain consistency in external morphology or behaviour are found to be distinct. Rarely do two varieties occur together in the same habitat or in the same season. It is for this reason that the status of var. *mysorensis* of *A. stephensi* is being considered only as that of a synonym. However, even though the two forms of *A. stephensi* occur in the same region there seems to be a distinct difference in the habitat, one being a breeder in man-made breeding places while the other is largely a breeder on the natural waters. The problem of *A. stephensi* has not yet been satisfactorily settled.

The official rules of zoological nomenclature recognise only genus, species and subspecies as valid names. Ranks below the subspecies, such as race, variety and strain, are not recognised.

What constitutes the difference between a subspecies and a species? Mayr (1977), who has made a special study of the problem of speciation, defines a subspecies as "an aggregate of local populations of a species inhabiting a geographic sub-division of the range of the species and differing taxonomically from other populations of the species." However, sometimes subspecies is defined as "a sub-division of a sexual species with all the attributes of a species except that there is a partial reproductive isolation."

Mating experiments alone can precisely differentiate between a species and subspecies. But such experiments are rarely done and often not possible because of difficulties of colonization. However, nowadays artificial mating techniques are available which can be used. The present day taxonomists are reluctant to create subspecies unless there is adequate proof.

A variety is nothing but a convenient terms to identify a morphological variant before it could either be named a subspecies or treated as a synonym. *A. gigas* and its varieties, for example, occur in such widely separated regions that for all practical purposes they are full species, but have not yet been so named.

In recent years several Indian anophelines, which had long been called varieties, have been raised to the status of species, such as *A. bengalensis, A. interruptus, A. ahomi,* and *A. nigerrimus.* In the absence of breeding experiments the naming of species and subspecies becomes rather arbitrary, depending upon the concepts of the author. Students of mosquito taxonomy are advised to read the excellent account by Reid (1968) as well as other publications such as by Bates (1949) and Mayr (1977). The publication of *Evolution—The Modern Synthesis* by Julian Huxley (1942) provided an inspiration to Senior White (1948) to reflect on this

subject, with particular references to the anopheline fauna of India.

First, dealing with the question of "biological races" or "crypto-subspecies", about the existence of which there was some evidence among Indian anophelines, he drew attention to a few pertinent statements of Huxley.

"Difference in ecological preferences may isolate groups as effectively as geographical barriers, often resulting in the production of "crypto-subspecies". Much of speciation is concerned with invisible, physiological characters".

"Groups may remain perfectly distinct though morphologically indistinguishable".

"Complete physical and genetical isolation may exist with slight or no character differences between the types".

"Certainly in most phyla, and probably in all, there exist groups or individuals which are distinct species in every sense except the accepted morphological one".

These statements undoubtedly lend support to the view that biological races can exist without any observable morphological difference. Such races have often been postulated in species such as *A. culicifacies, A. fluviatilis, A. stephensi, A. maculatus, A. philippinensis, A. sundaicus,* and *A. subpictus.*

Secondly, regarding mutations, Senior White cited the example of *A. sundaicus:* "A mosquito under optimum conditions has about 24 generations a year and following Huxley's postulate that some useful mutations which do not affect viability adversely would spread through a population in a little more than four years." Senior White suggested that it was such a mutation, possibly back to a recessive character long hidden in the genetic make-up, that enabled *A. sundaicus* in its invasion in the North Madras coast to invade fresh waters over 20 miles inland. Such a mutation was not available to the races present in Java where it failed to establish in less saline marshes further inland when it was driven out of the fish ponds by the classic work of Walch. This was because Java was in the very centre of the area of *A. sundaicus* distribution, while the East Coast of India is at its margin. This according to Senior White confirms Huxley's statement that the marginal zones of species are often characterised by a peculiar population-genetic structure or by special adaptations for primitive types which tend to be preserved near the margins of the range of a group.

Thirdly, geographic isolation and discontinuity are important factors which help in the development of fauna characteristic of some regions. Subspecies are most likely to arise where a species finds itself separated into isolated groups, as particularly occurs in oceanic faunas where decreased selection pressure permits increased variation. Senior White elucidated this matter, again selecting *A. sundaicus* as an example.

The tendency to form clines is illustrated by *A. maculatus.* According to Senior White, it may be found to have a cline for humidity tolerance extending from Malaysia to Central India and another cline for temperature resistance from Central India to sub-Himalayas. Even in Malaysia with its even climate the species is hetero-dynamic and there are months of greater prevalence.

Fourthly, Senior White also discussed the subject of rare species.

"Whilst abundant species will have greater evolutionary plasticity and a higher potency of adaptive change, rare species will tend to become subdivided into discontinuous groups which, once isolated, will have a greater likelihood of differentiating into separate species. Abundant species will differentiate into subspecies in different parts of a continuous range. Migration will keep distributing genes from one subspecies to its neighbours, and thus variability will be at its maximum."

He cited *A. gigas* and *A. lindesayi* as examples.

These and other observations of Senior White have remained speculative because no further attempts have been made to analyse the anopheline fauna of India from the evolutionary point of view. There is no doubt that such a study would yield very useful information not only regarding the differences in ecology but also those in vectorial capacity and amenability to control. For example, the probable disappearance of an anthropophilic race of *A. fluviatilis* in the Western Ghats but not of a zoophilic race in the Deccan, and the disappearance of *A. minimus* in the foothills of the Himalayas but not in Thailand and of *A. philippinensis* in the deltaic Bengal but not in Assam, can be attributed to the biological variations and effect of evolutionary changes.

Hybridization

Experiments of mating and cross-mating are the most important procedures used to determine the identity of species and subspecies. Such experiments are also very useful in identifying cryptospecies or sibling species. Some excellent reviews are available (Davidson *et al.*, 1967 and Kitzmiller, 1976). Most notable among the studies of this nature have been those on the *A. gambiae* complex of Africa. Similar studies have also been made on the taxonomic status of a number of populations in the North American *Anopheles punctipennis* complex (Byran, 1970 and 1973). Some Asian anophelines such as *A. stephensi*, members of the *'hyrcanus'* complex, and *A. superpictus* have also received attention (Colluzi, 1973).

Males of *A. stephensi* could inseminate females of *A. superpictus* but reciprocal crossing had to be made by forced copulation. In the former case hybrid larvae survived to the fourth stage and in the latter the development did not go beyond the first stage.

Studies on the *A. gambiae* complex of mosquitoes in the African continent provide an excellent example of the existence of "sibling species". Though morphologically difficult to distinguish, they exhibit clearly defined sub-complexes in their behaviour, ecology and chromosome patterns. Students of anophelines should be familiar with the work on *A. gambiae*. Therefore, the following extract from the summary of an excellent article by G.B. White (1974) would be of great interest to all entomologists, even though working in India.

"*Anopheles gambiae* complex mosquitoes are present throughout tropical Africa and its offshore islands. Recent work has shown that at least 6 cryptic or 'sibling' species are involved. They comprise 2 salt water species, *A. melas* and *A. merus*, 3 fresh water species—provisionally known as species A, B and C, and a mineral

water species known as species D. Artificial interspecies crosses yield sterile hybrid males. Rarity of hybridization in nature proves the reproductive isolation and valid genetic barriers between these 6 species".

"Morphological identification of most individuals of both salt water and the mineral water species is possible for larvae and adult females, using meristic features and other variable dimensions and ratios. Differential identification of the 3 fresh water species relies almost completely on cytotaxonomic methods".

"Species A and B occur together in most areas, extending southwards to subtropical latitudes and eastward to Mauritius. Proportions of mixed A-B populations may depend directly or indirectly on relative humidity, with A favoured when nocturnal humidities approach saturation. Species B is often absent from areas of highest humidity, but thrives in relatively arid savannas and steppes. Species C and D have relict distributions. Both salt water species are coastal; *melas* in West Africa and *merus* in East Africa and larger islands except Zanzibar, *merus* also spreads inland".

"Apart from species C, which is always zoophilic, all members of the complex are proven or probable vectors of human malaria and filariasis, but with some wide contrasts in their levels of vectorial efficiency. Transmission of some arboviruses (Tataguine, O'nyong-nyong) is associated with species B, and perhaps species A also. Species B may transmit seteriasis of cattle; *melas* and *merus* may also carry enzootic filariae".

"Much of the confusing ecophenotypic plasticity of *A. gambiae sensu lato* is attributable to the differential biological characteristics of these 6 species with their dissimilar geographical distributions, behavioural contrasts and asynchronous population dynamics. Shifts in the species balance occur regularly between A and B and between fresh water and salt water populations. Species C does not interchange so much with B under natural conditions, but may survive at high densities after control of A or B".

"Additional versatility is caused by genetic polymorphism in some of the species, notably B. This species is the most widespread, and individual females tend to be either endophilic or exophilic, anthropophagic or zoophagic, early biters or late biters, and doubtless other alternatives, according to the arrangement of their floating chromosome inversions."

"Control measures have to be considered separately for each of the sibling species. House spraying with residual insecticides against endophilic species A is possibly sufficient to break disease transmission (assuming favourable response of ancillary vectors) and even to eradicate A completely. Efficacy of this method against other species in the complex is reduced by their exophily, which can be enhanced by behaviouristic avoidance due to the extremely low avoidance due to the extremely low threshold of irritability exhibited by *gambiae* s.l. adults in general. Genetic polymorphism of species B may lead to true behaviouristic resistance. Larvicidal control of species A and B is beneficial, but made operationally difficult by their tendency to utilise temporary breeding sites. Effectiveness of DDT and cyclodiene insecticides is further limited by the development of physiological insec-

ticide resistance by species A and B. Adequate control of both salt water species, and probably the mineral water species also, can be attained by antilarval measures".

"Prospects of reduction of malaria and filariasis where *melas, merus* and species A and D are principal vectors may be much better than many people imagine. Widespread prevalence and inherent resilience of species B represents and insurmountable public health obstacle at present. Continued research may provide new control methods for integrated use against these mosquitoes. Concepts such as seeding breeding sites with pathogenic miscrosporidians or fungi, releasing sterile hybrid males or chemosterilized males, and other even more elaborate genetic control techniques, may be of special relevance to control of *A. gambiae* complex mosquitoes and diseases they transmit".

G.B. White (1975) has applied the following pre-existing Latin names to the members of the *gambiae* complex hitherto designated by alphabets:

Species A: *gambiae* sensu stricto, i.e., *gambiae* Giles, 1905;

" B: *arabiensis* Patton, 1905; and

" C: *quadriannulatus* Theobald, 1911.

No name has been given to species D.

This rather longish extract from White, it is hoped,would stimulate similar work on Indian anophelines.

A. stephensi has also provided material for genetical studies because of the ease with which the species can be colonized in the laboratory, and because of the interest arising out of the postulation of the existence of two varieties, designated *A. stephensi* type form and *A. stephensi* variety *mysorensis* (Sweet and Rao, 1937; Rao *et al.*,1938) on the basis of differences in length and width of eggs, length of the egg float and the number of ridges on the egg float. Experiments by Sweet *et al.* (1938) indicated that some of these characters bred true and exhibited dominance or recessiveness in the hybrids and recombinations in succeeding inbred generations. The two so called races have also been compared in respect of many other morphological characters and behavioural features. It has been suggested that the taxon, *A. stephensi,* is a species complex similar to the complexes of *A. maculipennis* and *A. gambiae.* Sweet and associates did crossing experiments which lent credence to their hypothesis; a certain degree of incompatibility was noticed. However, hybridisation experiments by Davidson and Jackson (1961) and Zuluetta *et al.* (1968) have shown that crosses between the two variants yield fertile offspring.

Rutledge *et al.* (1970) made a comparative study of three geographic strains of *A. stephensi* from India, Iran and Iraq. They studied differences in egg structure, fecundity, feeding behaviour, susceptibility to *Plasmodium cynomolgi,* and longevity. Reciprocal genetic crosses of three strains were made and fecundities of these parental crosses and all the six, types of F_1 hybrids were determined and compared with those of the three parent strains. Although increased fecundity was observed in certain parental crosses, reduced fecundity was also observed in others. Similar effects were also observed in F_1 hybrids. Discussing their result, Rutledge *et*

al. concluded, "It seems unlikely that cryptic species exist within the taxon *A. stephensi,* for interbreeding is possible among all forms tested thus far". In addition they state that "subspecies status for the type form and variety *mysorensis* seems inappropriate since these two forms are sympatric. On the basis of the present information it seems best to record these as local population variants". This has led to the *mysorensis* from being given the status of a mere synonym in the latest *Catalogue of the Mosquitoes of the World* by Knight and Stone (1977) while in the previous edition by stone *et al.* (1959) it was given the status of a subspecies.

Nevertheless, behaviour differences between the urban type form and the rural *mysorensis* form are so marked that further investigations are necessary to unravel this problem. They do not appear to be fully sympatric because the type form breeds in wells and cisterns while the *mysorensis* breeds in natural breeding places even in the same general area.

It is now well known that the *A. hyrcanus* group consists of several species (Reid, 1965) including *A. sinensis.* Crosses between different geographical strains of *A. sinensis* (Kanda and Oguma, 1970, 1972a and 1972b) were found to be fertile, but the hybrid chromosomes were partially asynaptic. One strain of *A. sinensis* from North Hokkaido (Japan) was unrelated to all other strains(Oguma and Kanda, 1972). These studies suggested that there may be distinct geographical races of *A. sinensis.*

Hybrids between *A. sinensis* and *A. lesteri* (another member of the *hyrcanus* complex) from Korea and Japan did not develop beyond pupal stage and the larvae were found to have asynaptic autosomes (Kanda and Oguma, 1972a). These studies have confirmed the species identity of forms which had been separated purely on morphological characters. There does not, however, seem to have been any studies on *A. nigerrimus,* the most common member of the *hyrcanus* group in India.

The study by Darsie and Cagampang—Ramos (1973) showed that reciprocal crosses by the forced mating technique between *A. subpictus* and *A. litoralis* from Indonesia were successful (52 to 86 per cent partial hatch in the F_1); but the F_1 males did not produce offspring. A back cross to *A. litoralis* males gave only a partial hatch (35 per cent). The F_1 morphologically resembled *A. subpictus.* Again the distinctiveness of two species, previously determined by minor morphological variants, has been confirmed by hybridization experiments, relating to behavioural differences, especially in breeding habits.

Hybridization attempts have been made with three anophelines *maculatus, stephensi* and *tessellatus,* by using induced copulation (Narang *et al.*,1972a). The *stephensi* × *maculatus* produced offspring, though only 6 out of 54 females produced embryonated eggs. Out of 371 eggs, 128 were embryonated and 48 of them hatched. 36 adult female (no males) emerged. None was fertile and all had reduced ovaries with none developing eggs. The *stephensi* female × *tessellatus* male cross produced about 45 per cent embryonated eggs of which only one hatched and that, too, died before reaching the fourth stage. The *tessellatus* × *maculatus* cross produced no embryonated eggs in either direction. The results indicated despite considerable genetic affinity between *stephensi* and *maculatus* but complete genetic isolation

between them. *A. tessellatus* seems to be closer to *stephensi* than to *maculatus*. The workers concluded that these hybridization results support the current classification within the subgenus *Cellia*.

Recently, Miles (1981) has shown that reciprocal crosses between two cytologically identifiable sibling species of *A. culicifacies* exhibited unidirectional male hybrid sterility.

There is urgent need to undertake such hybridization experiments among the anophelines of India to unravel the existence of sibling species and also to explain the known geographical variations in behaviour among such species as *A. fluviatilis, A. culicifacies, A. sundaicus, A. philippinensis,* and *A. subpictus*.

Cytogenetics

Chromosomes of mosquitoes have been known for a long time. Early studies by Steven (1908, 1910) determined the basic chromosome number as 6. The main interest at that time was in the basic chromosome numbers and the behaviour of chromosomes in mitosis and meiosis. It is only since 1944 or 1945 that the study of mosquito chromosomes has taken a leap forward after the description of the salivary gland chromosomes by Frizzi (1947).* Since then the work of cytotaxonomists such as Davidson, White, Kitzmiller, Colluzi, Frizzi, Rishikesh, Avirachan, Chowdaiah, Seetharam, Sharma, the two Narangs and others has expanded our knowledge considerably. The earliest studies on chromosomes of Indian anophelines was by Rishikesh (1955, 1959a, 1959b) who described the chromosomes of salivary glands of the larvae of *A. stephensi* and the chromosomes in spermatogenesis.

Karyotypes: All anophelines so far studied, including those occurring in India, have a diploid chromosome number of six. The three pairs of chromosomes are individually recognisable and are designated I, II and III, based on their length and the position of the centromere. The smallest pair, which is the sex chromosome, is designated as I and the remaining two longer pairs of mediocentric chromosomes as II and III. The latter two also differ slightly in their length.

Avirachan *et al.* (1969) described and illustrated the mitotic chromosomes of six species of Indian anophelines, viz., *A. barbirostris, A. nigerrimus, A. fluviatilis, A. stephensi, A. vagus,* and *A. subpictus*. Fourth instar female larvae of these species were used and chromosome preparations were made from brain tissue. The karyotypes were illustrated and a diagrammatic representation of karyotypes was also made (Fig.2). Though the chromosome number had been constant there were variations in their measurements and also in the position of the centromere. Chromosome I is sub-metacentric in *A. nigerrimus, A. fluviatilis, A. stephensi* and *A. vagus,* sub-telocentric in *A. barbirostris* and acrocentric in *A. subpictus*. Karyotypes of *A. tessellatus, A. subpictus, A. maculatus* and *A. annularis* as well as the entire process

*The earliest references to salivary gland chromosomes are Bogojawlenski (1934) and Sutton (1942) from information kindly furnished by Prof. Chowdaiah.

of mitosis have subsequently been described by Narang *et al.* (1972a) (Fig.2).

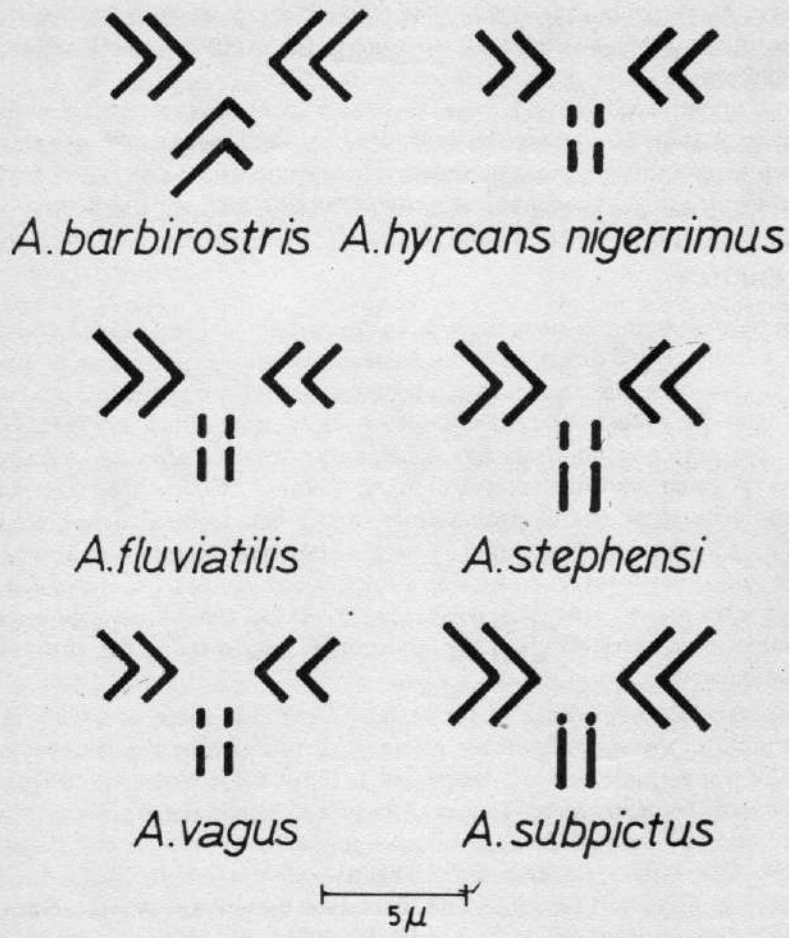


Fig. 2. Karyotypes, diagrammatic, of six species of Indian anophelines (Avirachan *et al.*, 1969). Courtesy: Dr. B.N. Chowdaiah.

The karyotype of *A. culicifacies* has been described by Aslamkhan and Baker (1969). Saifuddin *et al.* (1978) extended the studies on that species to polytene chromosomes using ovarian nurse cells of female adults. The karyotype consists of one pair of short subtelocentric sex chromosomes and two pairs of long autosomes, one being submetacentric and the other metacentric. Slight variations from their normal patterns have been noticed. Chowdaiah and associates have described and illus-

trated karyotypes of several Indian anophelines (Fig. 3).

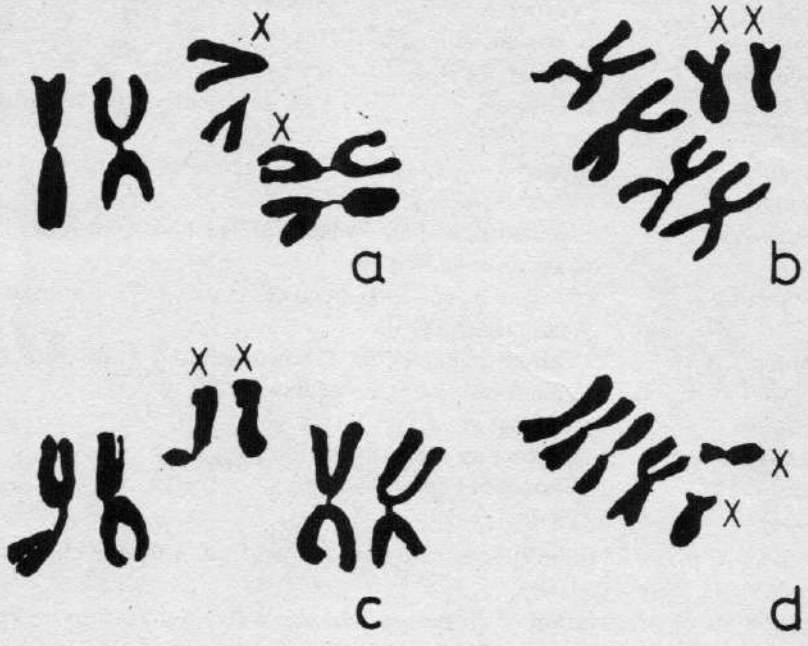


Fig. 3. Chromosome complexes, metaphase, of four species of *Anopheles*, re-drawn from illustrations by Dr. B.N. Chowdaiah and colleagues.
a—*A. subpictus* b—*A. fluviatilis* c—*A. barbirostris* d—*A. nigerrimus*

Salivary gland chromosomes: Salivary gland chromosomes, or polytene chromosomes, are quite large when compared with mitotic chromosomes. They are long and sinuous and exhibit a series of well stained bands and puffs, the serial arrangements of which are characteristic of each species or group of species. The bands and puffs can be assigned to definite loci on the chromosomes. Their arrangement and variations can provide useful clues to evolutionary relationships of species and group of species.

After Frizzi's (1947) description of the salivary gland chromosomes in *A. maculipennis*, extensive and very advanced work has been done on them in many species of anophelines. It has been possible to demonstrate the existence of sibling species, as for example in the *A. gambiae* complex already referred to. The possibility of accurate cyto taxonomic identification of crypto-species has opened up a new and practical field for clarifying geographic, seasonal and ecological distribution of the anopheline species (see Kitzmiller (1976) for further details).

Maps of salivary gland chromosomes have been published in a wide variety of anophelines; Indian species which have received attention being:

A. annularis	(Varma and Sharma, 1981)
A. barbirostris	(Chowdaiah *et al.*, 1970)
A. bengalensis	(Kanda, 1969)
A. culicifacies	(Saifuddin *et al.*, 1978). More recently, also by workers of Malaria Research Centre, Delhi.
A. fluviatilis	(Sharma *et al.*, 1968a, Chowdaiah and Seetharam, 1968a)
A. maculatus	(Narang *et al.*, 1973b)
A. nigerrimus	(Seetharam and Chowdaiah, 1971; Chowdaiah *et al.*, 1967) (Baker *et al.* 1968)
A. pulcherrimus	(Sharma *et al.*, 1969, Amir Khanian, 1973, Siddiqui and Aslamkhan, 1973)
A. subpictus	(Narang *et al.*, 1973a, Chowdaiah and Seetharam, 1973, Seetharam and Chowdaiah, 1974)
A. subpictus	(Narang *et al.*, 1974)
A. splendidus	(Sharma *et al.*, 1968d)
A. vagus	(Chowdaiah *et al.*, 1976)
A. varuna	(Pashan, 1973)

In respect of many of these species complete maps of the polytene chromosomes are available.

For methods of preparation of chromosomes, see WHO publication on entomological techniques (1975).

Narang *et al.* (1973) made a comparative study of the banding patterns of salivary gland chromosomes in the subgenus *Cellia.* The study of chromosomes of *A. maculatus* showed important evolutionary homologies with other members of the subgenus, including *A. gambiae, A. subpictus, A. pulcherrimus, A. tessellatus* and *A. stephensi.* These could be distinguished by means of the distinctive banding patterns of the X-chromosomes. Such a study also promotes an estimate of the kind and degree of cytogenetic changes that might have taken place during the evolution of these species. They presented evidence for arm exchanges, paracentric inversions and pericentric inversions.

In a series of papers Chowdaiah and colleagues have described the polytene chromosomes of several Indian anophelines (Chowdaiah *et al.*, 1967, 1970, Seetharam and Chowdaiah, 1974; Chowdaiah and Seetharam, 1975). Sharma *et al.* (1969) described the salivary gland chromosomes of *A. nigerrimus.* Figure 4 is from a microphotograph kindly provided by Dr. (Miss) K. Vasantha.

In general, in salivary gland preparations of anophelines, the chromosomes appear as 5 elements representing 3 pairs of chromosomes; a short telocentric X-chromosome and 2 pairs of long metacentric autosomes with arms of distinguishable lengths. The autosome arms are designated as 2,3,4,5 (2R, 2L, 3R, and 3L). All the chromosome have a banding pattern on them which can be analysed in detail. The patterns are characteristic of each species and of varieties.

Descriptions of the ovarian and salivary gland chromosomes of a few Indian

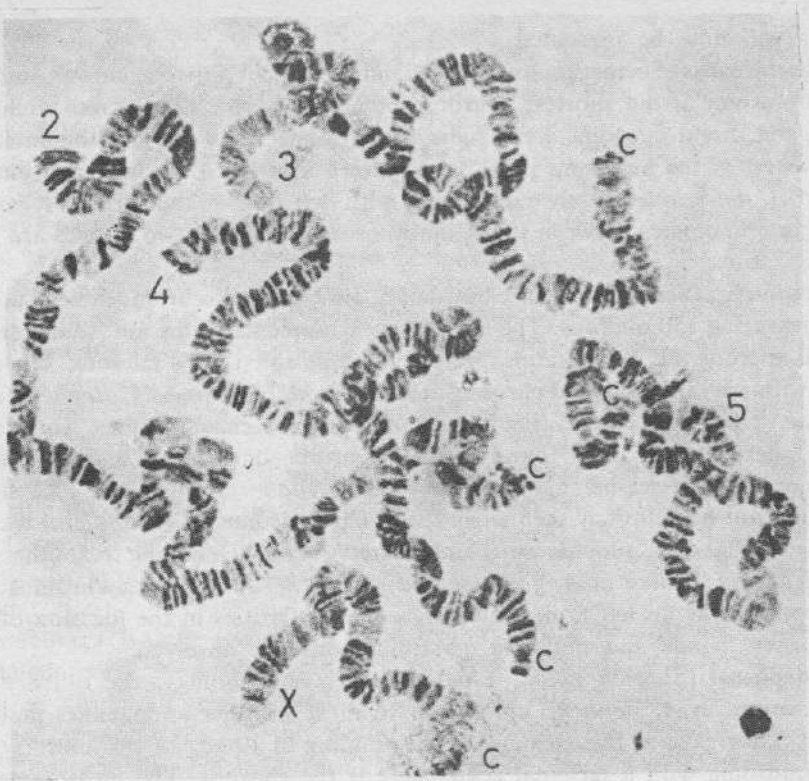


Fig. 4. Polytene chromosomes from ovarian nurse cells of *A. stephensi*. Numbers indicate chromosome arm designations. C = Centromere. Courtesy: Dr. (Miss) K. Vasantha.

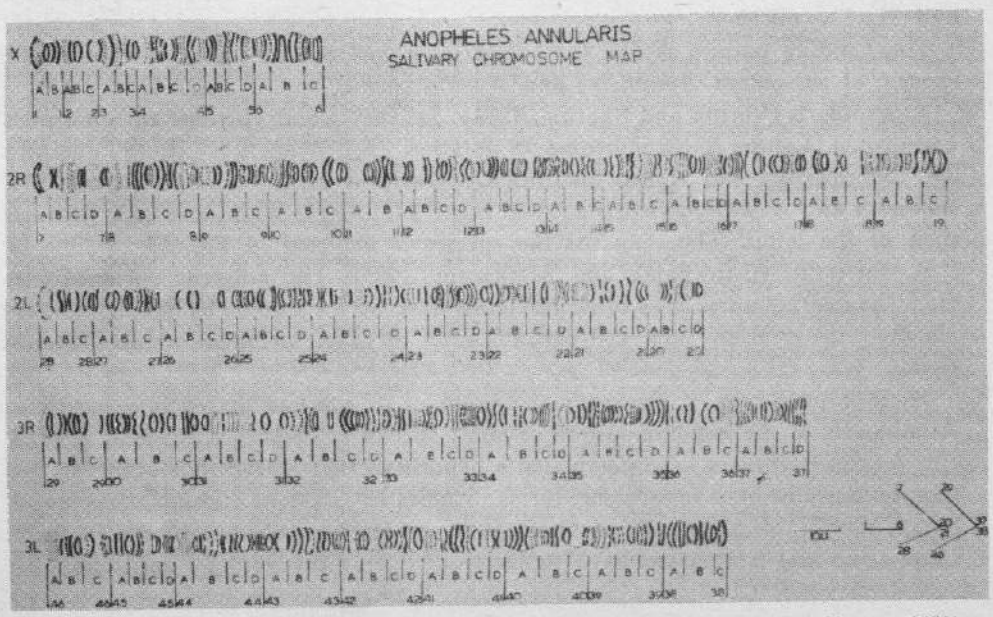


Fig. 5. Standard map of salivary gland chromosomes of *A. annularis*. (Varma and Sharma, 1981).

species will now be presented.

A. barbirostris (Seetharam and Chowdaiah, 1971): In polytene chromosomes, the X-chromosome is the shortest. Chromosome 2 has arms of approximately equal length and in chromosome 3 the right arm is longer than the left, the latter being the shortest of the autosome arms. The pattern in a few areas in the chromosome map of *A. nigerrimus* closely corresponds with two areas in the map of *A. barbirostris.* The homologies between the X-chromosomes of these two species are unmistakably clear.

A. subpictus (Seetharam and Chowdaiah, 1971): The X-chromosome is the shortest, measuring 130 microns. The average measurements of the autosomal arms are 2R 350 microns, 2L 180 microns, 3R 264 microns and 3L 284 microns. When compared with the salivary gland chromosomes of *A. pulcherrimus, A. stephensi* and *A. gambiae,* it has been very difficult to homologise the chromosomes. Some significant similarities have been found in the X-chromosomes of *A. subpictus* and *A. gambiae,* both species belonging to the series *Pseudomyzomyia.* But such similarities could not be definitely seen when the X-chromosome of *A. subpictus* was compared with that of *A. pulcherrimus* and *A. stephensi* which belong to another series, viz., *Neocellia.* Unlike in *A. gambiae* and *A. pulcherrimus,* the X-chromosome in *A. subpictus* has no left arm. However, some similarities in the location of a few dark heavy 'bands and puffs' in certain regions are observed.

A. stephensi (Sharma *et al.*, 1969): In gross morphology, the salivary gland chromosomes in *A. stephensi* are similar to those in other anophelines previously studied. They exist as five elements corresponding to 3 pairs of chromosomes. The X-chromosome with a terminal centromere is the shortest. The measurements of length are:

X-chromosome 65 microns, 2R 270 microns, 2L 160 microns, 3R 220 microns, and 3L 230 microns.

The banding pattern is like that of *A. gambiae,* but dissimilal to that of the species of subgenera *Anopheles* and *Nyssorhynchus* so far studied.

A. annularis (Varma and Sharma, 1981): Salivary gland polytene chromosomes constituted: a short X-chromosome 75 microns, 2R 280 microns, 2L 190 microns, 3R 250 microns, and 3L 105 microns. Comparisons with the chromosomes of *A. stephensi* and *A. philippinensis* showed more homologies with those of the former than of the latter. The map for the species is depicted in Fig. 5.

A. fluviatilis (Chowdaiah and Seetharam, 1975): The polytene chromosomes from the ovarian nurse cells of *A. fluviatilis* have been found to be like the salivary gland or the ovarian nurse cell chromosomes of other members of the subgenus *Cellia.*

There is no XL in *A. fluviatilis* as present in *A. gambiae* and *A. pulcherrimus.* Homologies of the banding pattern of many species of *Cellia* are not clear at present. As some workers have postulated a hypothesis that *A. fluviatilis* occurs in two morphologically indistinguishable forms. Studies on polytene chromosomes would be of interest to determine whether there are recognisable differences in them. Chowdaiah and Seetharam have so far studied only the strongly anthropophilic

forms, found in the hilly regions of South India.

A. culicifacies (Saifuddin *et al.*, 1978): The polytene chromosomes consist of five elements with a characteristic banding pattern, a very short X-chromosome with only one arm and 2R, 2L, 3R and 3L. The measurements are:

X-chromosome 79 microns, 2R 287 microns, 2L 157 microns, 3R 209 microns and 3L 223 microns.

Polytene chromosomes of *A. culicifacies:* Green and Miles (1980) have now studied the ovarian polytene chromosomes of *A. culicifacies* from India, Pakistan and Sri Lanka and have found two kinds of X-chromosomes to exist. One of these is identical with the map already published by Saifuddin *et al.* (1978). The other differs by having two inversions. No autosomal rearrangements have been noted, both types are found in natural populations. They have been tentatively called Xa+b+ and the other Xab; The former has been found in Pakistan and the latter in India and Sri Lanka and both types occur in Okhla near Delhi in the ratio of 23:3.

In the absence of heterozygotes, these findings are interpreted as evidence of two biologically distinct species within the taxon *A. culicifacies* Giles. The workers of the Malaria Research Centre, Delhi, have confirmed these findings and have extended the studies to several other areas in India (Dr. Subba Rao, personal communication).

A. tessellatus (Narang *et al.*, 1974): The complement of the chromosomes consists of a subtelocentric X-chromosome with one very short arm and two metacentric autosomes. No homologies in banding pattern with other members of the subgenus *Cellia* are apparent. The lengths of the polytene chromosomes are:

X 57 microns, 2R 178 microns, 2L 168 microns, 3R 225 microns and 3L 140 microns.

Chromosomal aberrations: Several types of aberrations have been found in the salivary gland chromosomes. Two of these, which have been extensively studied, are inversions and translocations. Inversions are said to occur when sections of individual chromosomes rotate 180° and the order of the arrangements of the sections of the chromosomes become inverted, such inverted chromosomes become stabilized in the population at certain proportion. If the inversion provides a better survival value they may even replace the normal arrangements in the population. Such inversions may lead to recognisable changes in the morphology and behaviour of the species.

Translocations are stated to occur when sections of one chromosome break away and attach themselves to other chromosomes. Such transfer of parts to other chromosomes may lead to partial or complete sterility. Often when there is partial sterility the strains of mosquitoes can be mass reared and used for genetic control of mosquito populations.

Both inversions and translocations may be found to occur in nature and may also be induced by radiation, chemicals and other methods.

Though several types of induced chromosomal aberrations have been described in culicines, there are only a limited number of aberrations reported hitherto in anophelines. Among anophelines of India, one translocation in *A. stephensi* had been

noticed. However, Baker *et al.* (1978) have described in *A. culicifacies* several types of aberrations both in mitotic and polytene chromosomes including translocations and inversions, single and multiple. These studies support the hypothesis that only the short arm of the mitotic X-chromosome is present in the polytene chromosome complement and that the 2R, 2L, 3R and 3L of the polytene chromosomes are correlated with the longer submetacentric and metacentric chromosomes respectively. There is no evidence for crossing over between X and Y chromosomes. The authors had not observed any aberrations in normal unirradiated individuals. However, recently the workers at the Malaria Research Centre, Delhi, have found two translocations in a natural population of *A. culicifacies* (Dr. S. Subba Rao, personal communication).

Evolutionary homologies: In a comprehensive discussion on evolutionary changes in the banding patterns of polytene chromosomes in anophelines, Narang *et al.* (1973b) have shown important homologies in the chromosomes of *A. maculatus* with those of the other members of the subgenus *Cellia*, including *gambiae, subpictus, pulcherrimus, tessellatus,* and *stephensi.* All these may be distinguished by the banding patterns of the X-chromosome. The major chromosomal events, pericentric and paracentric inversions and a translocation, have evidently differentiated *maculatus* and *subpictus.* With regard to the difference between *pulcherrimus* and *maculatus* in chromosome 2, the banding patterns seem to have arisen chiefly by means of paracentric inversions in both arms. In chromosome 3, a combination of paracentric inversion and inter-arm exchanges appear to have taken place. The two closest species, autosomally, appear to be *maculatus* and *stephensi* which are quite similar except for 3R and part of 2R. Narang *et al.* (1972a) had found that F_1 hybrid larvae of *maculatus* and *stephensi* show almost complete asynapsis of all arms in spite of the obvious similarity of banding patterns.

Colluzi and Kitzmiller (1975) in summarizing observations, have pointed out that in the subgenus *Anopheles* the banding patterns of species belonging to *Arribalzagia, Myzorhynchus* and *Anopheles* series could be easily homologized. Very little variation has been found in the free ends of the autosomal areas in the whole of the subgenus *Anopheles.* Comparative studies have also been made of the members of the subgenus *Cellia,* between *Pyretophorus* and *Neocellia.* In this case "a translocation and a pericentric inversion appear to be involved together with paracentric inversions in the chromosomal changes but most of the banding pattern can be homologized" (Narang *et al.,* 1973a). No homologies of an obvious nature could be observed when the primitive species of *Neomyzomyia* were compared with *Pyretophorus* and *Neocellia.*

An important and fruitful field of study awaits cytogenetists who may look into the cyto taxonomic features of other Indian anophelines which present some baffling problems of variations in behaviour in nature.

Further, extensive studies on salivary gland chromosomes have revealed interesting characters which are related to morphological or behavioural patterns. There have been several reviews on the subject, the most recent and informative of which is by Kitzmiller (1976). A short review by Chowdaiah *et al.* (1971) also deals with the cytogenetics of Oriental species.

Formal Genetics

Morphological mutants: Information in regard to morphological mutants of anophelines lags behind that on *Aedes* and *Culex*. However, about 50 morphological variants have been listed in *A. stephensi* alone in Pakistan by Aslamkhan and colleagues (Aslamkhan *et al.*, 1972a and b, and Aslamkhan, 1973a, b & c). Some of these can be used as markers to study the modes of inheritance. Recently Sharma (V.P.) *et al.* (1977) have detected a recessive autosome 'colourless eye' mutant spontaneously occurring in a laboratory colony of *A. stephensi* in Delhi. The 'colourless eye' is expressed at the larval, pupal and adult stages. Still more recently Subba Rao and Adak (1978) have reported a larval colour mutant, green larvae, in *A. stephensi*.

Some of the recently detected mutants in laboratory colonies are:

A. stephensi	Colourless eye, (Sharma *et al.*, 1977), Green larva, (Subba Rao and Adak, 1978), Red eye, (Sharma *et al.*, 1979), Black-green larval mutant (Suguna, 1981), Green larval mutant (Suguna, 1981), Spot larval mutant (Suguna, 1981).
A. culicifacies	Rose eye (Sakai *et al.* 1977), White eye (Sakai and Baker, 1980), Golden body (Sakai *et al.*, 1981), Red Thorax (MRC Ann. Rep., 1980), Creamish larva (MRC Ann. Rep., 1980), White eye (MRC Ann. Rep., 1980)

As cytotaxonomy is rapidly advancing many more types of mutants are likely to be detected.

Bio-chemical markers: Some very significant information has been obtained by electrophoretic studies on enzymes, resulting in identifying bio-chemical markers, on species, such as *A. labranichiae, A. freeborni, A. punctipennis*, etc. But except for a few studies on *A. stephensi* strains in Pakistan, Indian anophelines have not received much attention. Enzyme markers would provide valuable information on the problems of speciation and intraspecific variation.

In a very recent study Miles (1979) has prepared a key for the different species of the *gambiae* complex using bio-chemical characters.

Studies on the association of morphological characters with the loci on chromosomes have been done with species of *Aedes* and *Culex*, but only insufficiently with *Anopheles*. Working with enzyme systems in *A. stephensi*, it was found that the loci for them were on autosomes. The loci for alcohol dehydrogenase (adh) have been assigned to linkage group II and Est to linkage group III. Such studies would be of great importance in investigations on the genetics of insecticide resistance. For example, Haridi (1973) has suggested that DDT and dieldrin resistance are found on the same linkage group in *A. gambiae*. Genetics of insecticide resistance are dealt with in Chapter 9.

Linkage: Certain morphological and behavioural features have now been associated with linkage groups of chromosomes. For example, the 'colourless eye' in *A. stephensi* has assigned to linkage group II and Green larva and Greenish brown larva

to linkage groups III (Subba Rao and Adak, 1978 and 1981). Two more morphological mutants, Green and Spot, have been shown to be linked while they segregated with Black larva (Suguna, 1981).

In *A. culicifacies* species A, linkage group co-ordination has been made using 3 sex chromosomes—autosome translocations and four genetic markers (Sakai *et al.*, 1978) while in species B Red thorax and Greenish larva have been assigned to different autosomal linkage groups (Malaria Research Centre, Ann. Rep., 1981).

Sex genetics: The work of Aslamkhan (1973a) seems to indicate that in *A. stephensi*, sex determination is of the XY type. Earlier Mason (1967) had shown a similar system for *A. gambiae*. Additional support to the XY types is provided by the work of Narang and Kitzmiller (1972) on the esterase polymorphism in a normal population of *A. punctipennis*.

Behavioural genetics: There is reason to believe that certain aspects of mosquito behaviour are controlled by genetic factors. In *A. stephensi*, for instance, it is now known that chromosomal inversions are associated with differences in behaviour. With reference to one type of inversion (Karachi or KR), White (1974b) has listed the difference based on the observations of several authors:

Karyotype	*ST/ST*	*ST/KR*	*KR/KR*
Egg length	Long (*stephensi type*)	Intermediate	Short (*mysorensis*)
Larval survival under crowding	Inferior	Intermediate	Superior
Larval growth rate	Very slow	Fast	Very slow
Time of pupation (day-time)	Early	Intermediate	Late
Adult longevity	Inferior	Superior	Inferior
Hetero-specific mating activity	High	Intermediate	Low
DDT tolerance	Inferior	Superior	Superior
Circadian flight activity	Early	?	Late
Responsiveness to light	Greater	?	Lesser

Genetics of susceptibility to infection: Though many experiments have been conducted regarding the susceptibility of culicines to filaria and malaria infections, both human and animal, very little has been studied in respect of anophelines except some gross studies. Differences in susceptibility to human malaria infections are well known, but the genetical aspects still remain to be explored.

Genetics of insecticide resistance: Brown and Pal (1971) have given an exhaustive review of insecticide resistance of susceptibility in arthropods and included two chapters dealing with mosquitoes. This subject will be dealt with separately in a different section (see chapter 9).

Genetic control: Genetic control and related studies have been largely concerned with culicines, particularly *Culex fatigans* and *Aedes aegypti*. Anophelines, especially in India have so far received very little attention. The only field trial of some magnitude on anophelines was that conducted in El Salvador against *A. albimanus* using chemosterilization of males. The experiment seems to have reduced the natu-

ral populations to very low levels and completely prevented the usual autumnal peak (Weidhaas *et al.*, 1974 and Lofgren *et al.*, 1974). There is no recorded instance in anophelines of the type of cytoplasmic incompatibility which has been noticed in certain strains of *Culex fatigans* (Laven, 1967).

The possibilities of genetic control of mosquitoes received very early attention in India at the National Institute of Communicable Diseases. A few laboratory and field studies were made (Krishnamurthy *et al.*, 1962; Ramakrishnan *et al.*, 1962). But it was under the ICMR/WHO Research Unit on the Genetic Control on Mosquitoes that an extensive laboratory and field trials were carried out on *Culex fatigans* and *Aedes aegypti* during a period of six years (1970 to 1975) near Delhi. Many papers dealing with both the basic and applied aspects of genetic control, have been published on such aspects as cytoplasmic incompatibility, chemical and radiation sterilization, population ecology and genetics, translocated strains and their use in the field, field experiments on control etc. They are too numerous to be mentioned here. However, no large scale field experiments were carried out on anophelines. [Ramachandra Rao (1974) for a Summary].

The whole field of genetics and cytogenetics of anopheline is still in a preliminary stage and there are high prospects of the knowledge of these subjects being useful, even essential, for not only solving many taxonomic problems but also for understanding the behaviour and control of mosquito populations.

Anophelines as Vectors of Human and Animal Disease

Anophelines are well known as the only vectors of human malaria all over the world wherever the disease occurs. They are also known to be vectors of several species of primate malaria and of malaria of a few other animals. They are known to play a part in the transmission of human and animal filariasis. Their importance in the transmission of certain arthropod-borne viruses has been recognised.

In a strict sense, malaria plasmodia, filaria worms and arboviruses could also be listed along with other parasites of mosquitoes mentioned in a previous chapter. However, they are usually not regarded as such mainly because they do not seem to have any adverse effect on the mosquito host. In fact, the mosquito host is an essential feature in their life cycles. Heavy infections of filaria worms may, however, affect the infected mosquitoes.

Human Malaria

Out of about 365 species of *Anopheles* only about 70 have been known to have any significant role in transmission of human malaria. The following list includes practically all anophelines which have been recorded as vectors either as major ones or of local and limited importance. Those which are regarded major vectors are indicated by an asterisk.

Species	*Main Zone of Distribution*
*aconitus**	Oriental region.
*albimanus**	Central and South America.
albitarsis	Central and South America, Caribbean.
annularis	Oriental region.
*aquasalis**	Central and South America, Caribbean.
argyritaris	Central and South America, Caribbean.
*atroparvus**	Europe (largely littoral).
aztecus	Mexico,
baezai	Malaysia, Thailand, Indo-China, Philippines, Indonesia.
*balabacensis**	Oriental region.
bancrofti	Australia, New Guinea, Bismarck Islands.
barbirostris	Oriental region.
*bellator**	South America, Caribbean.
brunnipes	Africa.
*campestris**	Malaysia.
*claviger**	Europe, West Asia, North Africa.
cruzii	Brazil, Venezuela.

*culicifacies**	Oriental region.
*darlingi**	Central and South America.
dirus	Thailand.
dthali	West Asia, North Affica.
dureni	Congo, Ivory Coast.
farauti	New Hebrides, Australia, Solomons, Bismarck Islands. New Guinea, Moluccas, Santa Cruz Islands.
*fluviatilis**	Oriental region and West Asia.
freeborni	Western United States, Mexico, South Western Canada.
*funestus**	Ethiopian region
gambiae and several members of the complex*	Ethiopian region.
hancocki	Ethiopian region.
hargreavesi	West Africa.
hispaniola	Mediterranean region.
*indefinitus**	Oriental region.
jeyporiensis var. *candidensis**	Oriental region.
karwari	Oriental region.
kochi	Oriental region.
koliensis	Solomon Islands, New Guinea.
*labranchiae**	North and South Mediterranean region.
*letifer**	Malaysia, Sumatra, Borneo.
*leucosphyrus?**	Oriental region.
*maculatus**	Oriental region.
maculatus var. *willmorei*	Oriental region.
*maculipennis**	Continental Europe, South-West Asia to Persian Gulf.
messeae ssp of *maculipennis*	Northern Europe.
mangyanus	Philippines.
*minimus**	Oriental region.
minimus var. *flavirostris**	Philippines, Kalimantan, Java.
moucheti	Africa.
multicolor	North Africa, Cyprus, Middle East, Pakistan, Afghanistan, India.
nigerrimus	India, Sri Lanka, Burma, Thailand, Malaysia, China, Indonesia.
nili	Ethiopian region.
*nuneztavori**	Venezuela, Guianas, Brazil, Bolivia, Colombia.
pattoni	China (mainland).
*pharoensis**	Egypt, Ethiopian region, Israel, Syria, Yemen, Saudi Arabia.
*philippinensis**	Oriental region.
*pseudopunctipennis**	Antilles, Southern United States to Argentina.
punctimacula	Central America, South America, Caribbean.
punctulatus	New Guinea, Bismarck Islands, Solomon Island, Moluccas.
*quadrimaculatus**	North America.
rufipes	Ethiopian region.
*sacharovi**	Europe, West Asia.
sergentii	North Africa, West Asia, Pakistan.
sinensis	Oriental region.
splendidus	India.
*stephensi**	West Asia, Afghanistan, India, Burma and Pakistan.
stephensi var. *mysorensis**	India, Pakistan, Iran.
subpictus	Oriental region.

*sundaicus**	Oriental region.
*superpictus**	Mediterranean region, West Asia, U.S.S.R., Aghanistan, Pakistan.
tessellatus	Oriental region, Moluccas, New Guinea, Maldive Islands
umbrosus	Oriental region.
varuna	India, Sri Lanka, Burma, Nepal.

In the preparation of the above list, the list provided by Russell *et al.* (1963) has been useful and has been modified on the basis of additional information now available.

The species of *Anopheles* in India which have a role in human malaria transmision are:

Species	*Spheres of influence*
A. culicifacies	Extensively all over India, but becoming weaker proceeding towards east. It is pre-eminently a vector in the plains of India.
A. fluviatilis	All along the Western and Eastern Ghats, in the Deccan, and central and east central India and all along the foothills of the Himalayas.
A. minimus	All along the foothills of the Himalayas and in the eastern region of the country, i.e., Assam and neighbouring hill States.
A. stephensi	In many cities and towns of India. Its variety *mysorensis* is also a vector in many rural areas in the Deccan and east central India.
A. philippinensis	In the deltaic areas of Bengal, Assam and neighbouring hill States such as Meghalaya.
A. balabacensis	Only in the hill regions of eastern India.
A. annularis	Assam, Bengal, coastal Orissa. (Also Nepal)
A. sundaicus	Coastal Bengal, coastal Orissa, coastal Andhra Pradesh and the Andamans.
Purely local vectors are:	
A. varuna	In scattered localities in east central India, in Kerala and perhaps in Bengal and in Lakshadweep also.
A. aconitus	In scattered localities in east central India, Orissa and Assam.
A. jeyporiensis var. *candidiensis*	In scattered localities, in Kerala, Karnataka, east central India and Assam.
A. maculatus	In scattered localities in Assam and Meghalaya.
A. tessellatus	Lakshadweep Islands (on epidemiological grounds).
A. subpictus	Tamil Nadu and Pondicherry.

Natural infections have also been found on stray occasions in a few other species such as *A. vagus, A. karwari, A. kochi, A. ramsayi, A. splendidus* and *A. pallidus.* But their importance as effective vectors remains questionable. Detailed information on the vectorial status of different species is given in the sections dealing with them. *A. tessellatus* is, however, regarded as a vector in Lakshadweep solely on epidemiological grounds.

It is of interest to note that though a very large number of dissections have been made of a few common anophelines, such as *A. barbirostris, A. nigerrimus, A. jeyporiensis, A. sinensis* and *A. tessellatus,* no infections have been found in them in India. But some of them have been found infected in other countries, e.g., *A. tessellatus* in Maldive Islands, (where it was the sole vector, till it disappeared after DDT spraying), Hong Kong, Indo-China and Kalimantan; *A. barbirostris in* Malaysia and Sulawesi; *A. nigerrimus* in Malaysia and Indo-China (stray infections); *A. sinensis* in China, Indo-China and Indonesia; *A. jeyporiensis* in Hong Kong, South China, Indo-China and Burma. *A. subpictus* is a vector in Indonesia.

The detailed lists of natural infection among the anophelines of the world given by Horsfall (1972) and the lists of dissections of Indian anophelines given by Wattal (1961) for non-vector species and by other authors for ten vector species in *Vectors of Malaria in India* (1961) are most useful. There have also been earlier lists by Covell (1931a) and others. No attempt has been made in this volume to present such detailed lists. However, major records of natural infections found in India are referred to in the special section dealing with individual species.

What makes a species a vector and another not is a question which has intrigued malariologists for a long time. There are many factors which have to be considered in this connection.

(i) **Susceptibility to infections:** The species has to be susceptible to infection with the human malaria parasites. A large number of investigations have been done and though it is recognised that most species of *Anopheles* can be infected with human malaria parasites under appropriate laboratory conditions, there are real differences in the degree of susceptibility. Some species are easily infected, while others are not.

Early studies by Boyd and Stratman Thomas (1934) showed that *A. quadrimaculatus* was more susceptible than *A. crucians* to all three species of human plasmodia. Boyd and Kitchen (1936) in comparative studies found that *A. quadrimaculatus* and *A. punctipennis* were about equally susceptible to two strains of *P. vivax;* but *A. punctipennis* showed very variable susceptibility to strains of *P. falciparum* ranging from high susceptibility to complete refractoriness.

Roy (1943) compared the infection susceptibilities of *A. subpictus* (a salt water form) in Bengal with *A. stephensi* fed on the same donors. He found that both *A. subpictus* and *A. stephensi* could be infected, but in different degrees. In *A. subpictus* five gut infections were found in 15 mosquitoes infected with *P. vivax* and 16 gut and five gland infections in 50 mosquitoes infected with *P. falciparum.* With *A. stephensi,* however, he found 13 gut and 10 gland infections in 18 mosquitoes infected with *P. vivax* and 30 gut and 22 gland infections with *P. falciparum* out of 52 specimens dissected, showing that under the same conditions *A. stephensi* was more susceptible.

Russell and Mohan (1939c) carried out comparative susceptibility studies experimentally. They found sporozoite rates of 2.3 per cent in *A. subpictus,* 53.8 per cent in *A. culicifacies,* and 87.1 per cent in *A. stephensi;* showing that *A. subpictus* was poorly susceptible while *A. stephensi* was highly susceptible, while *A. culicifacies* occupied an intermediate position.

Recently, Das *et al.* (1979) in Salem City in Tamil Nadu found that when fed on the same gametocyte donors, *A. stephensi* females were infected upto 48 per cent, while no infection resulted in *A. subpictus.*

Bibikova (1975) has reviewed the literature (in Russian) on susceptibility of anophelines to strains of *Plasmodia* imported from different countries and found variations in the infectivity of the strains.

In Malaysia, Sandosham (1965), summarizing the finding of several experiments made by others, showed that nine species of subgenus *Cellia* were infected in the laboratories with an average infection rate of 27 per cent, the range varying from 6 per cent in *A. annularis* to 56 per cent in *A. vagus.* Some of the other infections were in *A. maculatus* 29 per cent, and in *A. kochi* 33 per cent. In the subgenus *Anopheles*, however, the average for eight species was only 7 per cent. As is obvious, these figures do not truly reflect the vectorial capacity in nature. For instance, *A. vagus* is a non-vector or only a poor vector, but it shows a high rate in laboratory infections. However *A. campestris* of the *barbirostris* group is a very efficient vector in nature, but poorly infected in the laboratory. These data bring out the fact that there are considerable variations in susceptibility of different species.

The comparative susceptibility status of anophelines to human malaria parasites can be best exemplified by the work of Barber and Rice (1935) with a few European anophelines. They found that 20 per cent of *A. sacharovi*, 20 per cent of *A. maculipennis*, and 55 per cent of *A. superpictus* could be infected. Similar observations have been made by many workers.

Russell and Mohan (1939d) were not able to show any difference in the susceptibility of the two races of *A. stephensi.* They also (1939c) showed that the medium in which the larva had developed—whether salt water or fresh water, highly polluted water or plain water—did not alter the degree of susceptibility. But the type form has been regarded as a better vector than *mysorensis.*

Susceptibility of anophelines to both *vivax* and *falciparum* infections has been amply demonstrated. However, *P. malariae* has always been a difficult species to deal with. Siddons (1944a), however, succeeded in experimentally transmitting *P. malariae* through *A. culicifacies.* The time required for the extrinsic incubation period was considerably longer than with other species.

A very significant study on the degree of susceptibility of anopheline species to monkey malaria, *P.c. bastinealli* by Warren *et al.* (1963), has been dealt with in detail later under 'Monkey Malaria'. Its significance, is that while many of the species tested were capable of being infected, there was no evidence that they could be vectors in nature. Among the species they found (a) some which could be infected but only development of oocysts and not sporozoites occurred; (b) some in which infections developed to the sporozoite stage and (c) some of them could transmit the parasite to other monkeys. Only five species were regarded as having a sufficiently high degree of infection to enable them to be vectors in nature.

Is there a genetic basis for such differences? There are strong indications that there is such a basis. The very fact that the degree of susceptibility under same conditions

and with identical donors can vary so much supports the existence of innate differences. Such differences can exist even among different strains of the same species, perhaps adapted to the local strains of parasites themselves. Working on bird malaria, Huff (1934) showed that *Culex pipiens* could be selected for susceptibility or non-susceptibility to *P. cathmerium* and *P. relictum* in the laboratory. Frizzi *et al.* (1978) have shown that a genetic basis for susceptibility occurs in anopheline mosquitoes. However, there appear to be greater genetic differences among species than among strains of the same species.

The infections found in nature may also be influenced by the biological rhythms of the vector, the parasite and the host and their mutual adaptations. The adaptation of malaria parasites to their vectors in nature is a complex phenomenon. Hawking *et al.* (1966) have pointed out that the malaria parasites have a rhythm of 24, 48 and 72 hours according to species; but they have to be synchronised with the 24 hour rhythm of the vertebrate host. In addition, the 48 hour or 72 hour biting rhythm of the vector has also to be taken into consideration.

While on the subject of susceptibility to infections, one should not fail to take note that the quality and number of gametocytes also play a very important part. Generally speaking high concentrations of gametocytes produce more infections, but it is not always true (Knowles and Basu, 1943).

(ii) **Host preferences:** An adult *Anopheles* has to take blood meals on man at least twice, once when it becomes infected and later after about 10-12 days when it can infect another man. Quite naturally, if the species is one which takes human blood meals only rarely, the chances of such a second meal on man become rare. Species which feed on human blood regularly are the most likely to be the vectors. Most of the anophelines of India normally feed on animals, particularly cattle, in urban and rural surroundings; but they can also feed on other animals such as monkeys, deer, pig, etc., when in purely sylvan surroundings. Though many observations have been made on the host feeding habits in towns and villages, they have been made rarely in forests.

A. fluviatilis, A. minimus and *A. balabacensis* are species which feed preferentially on man and are therefore good vectors wherever they occur, but species like *A. culicifacies* and *A. annularis* feed predominantly on cattle. The anthropophilic indices of the former are generally of the order of 70-90 per cent and of the latter less than 10 per cent. However, what the members of the latter group lack in their habit of feeding on man is made up by their occurring in large numbers.

Species like *A. stephensi* are intermediate between the two in their man biting habits. In urban areas *A. stephensi* bites man in the absence or scarcity of cattle and it is a very efficient vector in such situations. The presence or absence of cattle, therefore, can be of critical importance. For example, in Ennore, north of Madras city, *A. culicifacies* has an anthropophilic index of about 80 per cent in the casuarina plantations where there are few cattle. *A. fluviatilis* and *A. stephensi* var. *mysorensis* of the Deccan plateau feed on cattle as does *A. culicifacies*. It would appear that there are two biological races of *A. fluviatilis;* one occurring in the hills and feeding predominantly on man, and other occurring in the plains and predomi-

nantly feeding on cattle. (*For details see section on 'Host selection'.*)

(iii) **Longevity:** The longer a mosquito lives, the greater are its chances of becoming a vector. It is known that once an anopheline is infected and develops sporozoites, it is capable of transmitting malaria practically for its life time. But there is some evidence that younger the mosquito the greater is its infecting capacity. Very few studies on actual longevity in nature have been made on Indian anophelines. The studies by Russell and Ramachandra Rao (1942b) on *A. culicifacies* came nearest to natural conditions and revealed an average mortality of 50 per cent every two days (re-calculated as 24 per cent per day by Macdonald) but about three per cent lived to the dangerous age of 10 days. Other observations made indirectly by Afridi *et al.* (1940) in Delhi also gave similar findings. (See Curtis and Rawlings, 1980 for very high longevity in Sri Lanka). There are, in addition, several observations made under laboratory conditions. Generally temperatures of 30°C and 75-80 per cent R.H. are the optimum combination for higher longevity. These are dealt with in the appropriate sections relating to each species.

In the case of other major vectors which are prevalent in humid forests or wooded areas, it has been surmised that a substantial number can live longer than 10 days, the time required for the extrinsic incubation of the parasite where conditions of temperature and humidity are favourable. That may be one of the reasons why species like *A. balabacensis* and *A. fluviatilis* can be very good vectors. Climatic conditions are, therefore, very important in determining the vectorial capabilities of anophelines. Those which have their seasons of prevalence during the monsoon and post-monsoon months are the most efficient. In Bangladesh, 31.3 per cent of *A. balabacensis* have been found live long enough to become infected.

(iv) **Density:** The density of the vector species at a given time is one of the most important factors in determining vectorial efficiency. Density is reflected in the number of females of the species biting man per night. Higher the density, the greater is the number of bites per person. With species with a higher human biting habit the numbers can be low, as with *A. fluviatilis* and *A. minimus*. But with a species with man-biting habit of a low order, the numbers have to be really high, as in the case of *A. culicifacies* or *philippinensis*. The role of vector densities in malaria epidemiology is discussed in another chapter.

The factors which contribute towards a species becoming a vector and determine its efficiency are many and varied. Apart from broad generalizations it would not be possible to state precisely what a species would do in any given area without detailed studies on the parameters referred to above. The finding of sporozoites in nature, a good evidence in many types of places with concentration of human population and malaria prevalence, should not by itself be regarded as final proof of a species being a vector because the mosquito may not have a chance to bite man again. As human infection experiments are not possible, the last of Koch's postulates, namely ability to transmit the infection to the particular host, cannot be clinched. One has to depend largely on circumstantial evidence, such as the place of collection, numbers of the species occurring and known to be biting man, actual occurrence of human malaria cases at the time, and morphology of the oocysts and

sporozoites, etc., to decide whether an infection is that of a human plasmodium. It is, however, of interest that except in a few cases, like *A. umbrosus* in Malaysia, the detection of sporozoites among the anophelines associated with man concurrently with periods of known malaria prevalence in a locality, have largely proved themselves to be of human origin. Control of such anophelines in many situations has also led to the successful control or elimination of the disease, providing indirect evidence.

Modern techniques of dissection are rapid and a team of two workers can easily dissect 200 mosquitoes a day. With certain species like *A. culicifacies,* they would have to dissect several thousand specimens before concluding that it is a vector. On the other hand, with species like *A. fluviatilis, A. minimus* and *A. balabacensis* a couple of hundred specimens may be adequate. No species should be eliminated as a vector until very large numbers have been dissected.

From a general study of the sporozoite rates, the malaria vectors in India may be graded in their order of importance as follows:

A. Efficiency as a vector	*B. As cause for number of Malaria cases*
A. fluviatilis	*A. culicifacies*
A. balabacensis	*A. fluviatilis*
A. minimus	*A. minimus*
A. stephensi	*A. balabacensis*
A. sundaicus	*A. stephensi*
A. culicifacies	*A. philippinensis*
A. philippinensis	*A. sundaicus*

Monkey Malaria

Anophelines are proven vectors of certain species of monkey malaria. Aberle (1945) referred to the many studies made by workers till then to infect mosquitoes with various species of monkey plasmodia. According to him, all attempts to infect mosquitoes other than anopheline species were unsuccessful, except one instance of the development of oocysts in *Culex vishnui.* Of course, all anophelines cannot be infected. Among the monkey plasmodia the most studied are *P. cynomolgi, P. inui, P. knowlesi, P. brasilianum, P. gonderi,* and *P. kochi.*

The major species of mosquitoes infected by the three species of monkey malaria which occur in India are:

	Natural infections	*Experimental infections*
P. inui	*A. elegans*	*A. stephensi*
		A. fluviatilis
		A. tessellatus
		A. elegans
P. cynomolgi	*A. elegans*	*A. maculatus*
		A. kochi

		A. culicifacies
		A. annularis
		A. vagus
		A. splendidus
		A. fluviatilis
		A. tessellatus
		A. elegans
		C. vishnui
P. knowlesi	unknown	*A. stephensi*
		A. annularis

[See below for work of Warren *et al.* (1963)]

Experimentally Jaswant Singh *et al.* (1949) succeeded in infecting *A. stephensi* and *A. annularis* with *Plasmodium knowlesi.* They also succeeded in transferring the infection to a fresh monkey by inoculation of infected glands. It may be noted that various attempts made in the past for finding the natural vector of *P. knowlesi* had been unsuccessful. Mulligan and Knowles obtained only one *A. stephensi* with oocysts in 400 attempts in experimental infection. (see Russell, 1946).

Jaswant Singh, Ray and Nair (1949) reported dissections of over 700 females of *A. culicifacies* which were given infective blood meals with *P. knowlesi* without any positive result.

In experimental infection studies in the Nilgiris, Choudhury *et al.* (1963a) found that *A. stephensi, A. fluviatilis, A. tessellatus,* and *A. elegans* were susceptible to mixed infections of *P. cynomolgi* and *P. inui* when fed on *Macaca radiata.* Successful transmission by bite to clean monkeys was obtained with *A. stephensi, A. fluviatilis* and *A. tessellatus.* Choudhury *et al.* (1963b) were also able to find *A. elegans* infected in nature in the same area. 10 out of 84 *A. elegans* females collected and dissected showed sporozoites, which were confirmed after staining. Eight others showed oocysts. Sporozoites from naturally infected *A. elegans* when inoculated into clean monkeys produced infection.

In an experimental study Collins *et al.* (1968) infected *A. maculatus, A. stephensi* and *A. balabacensis* with *Plasmodium fieldi* by feeding them on infected rhesus monkeys. Transmission by bite to fresh monkeys was also obtained in all the three species. *A. maculatus* had 2.3 oocysts per gut, *A. stephensi* 1.3 oocysts, and *A. balabacensis* 3.2 oocysts. The mosquitoes were held at 25°C and the extrinsic incubation period was 12 days.

In Sri Lanka, Nelson (1971) showed that sporozoites from naturally infected *A. elegans* when inoculated into a rhesus monkey resulted in an infection with *P. shortti.*

In an extensive series of studies made on susceptibility of 27 species of Oriental anophelines to *Plasmodium cynomolgi bastianellii,* Warren *et al.* (1963) found that a number of them demonstrated at least a moderate level of susceptibility. Perhaps the members of the subgenus *Anopheles,* except *A. lesteri,* were generally poor hosts, but several species in the subgenus *Cellia* were good hosts, including *A.*

maculatus, A. sundaicus, A. kochi, and *A. philippinensis.* Transmission was successfully accomplished in the laboratory with the five species mentioned above. Unfortunately because of insufficient numbers available, they were not able to study extensively the members of the *A. leucosphyrus* group which contains at least three proven vectors of monkey malaria, viz., *A. balabacensis, A. leucosphyrus* and *A. hackeri.*

A summary of observations of Warren *et al.* can be useful to students of Indian anophelines because several of the species occur in India. Therefore, they are given in some details below:

A. barbirostris	As compared with *A. maculatus* and *A. philippinensis,* the proportion susceptible was much lower. Though oocysts were formed, salivary gland infection did not develop.
A. campestris	Very low susceptibility.
A. donaldi	Quite refractory.
A. hodgkini	Poor infection.
A. hyrcanus and *A. nigerrimus*	Susceptibility not evaluated because of insufficient numbers.
A. argyropus	Virtually non-susceptible. Only 3 out of 108 mosquitoes became infected.
A. crawfordi	None among 5 fed became infected.
A. nitidus	Low level susceptibility. One specimen out of 46 dissected showed gut infection.
A. lesteri	Quite susceptible, but only a few of the mosquitoes dissected proved to have sporozoites. 21 out of 36 dissected had oocysts.
A. peditaeniatus	Extremely low level of susceptibility. Only two were found infected out of 110 dissected.
A. sinensis	14 out of 68 dissected were found to be infected.
A. letifer	Susceptible to infection upto the oocyst level, but its potential ability to transmit *Plasmodium cynomolgi* is practically 'nil'.
A. baezai	Refractory to infection.
A. roperi	Probably not susceptible to monkey malaria. This member of the *umbrosus* group is reported to have a role in transmission of human malaria. It was reported to have a natural infection rate of 11.4 per cent, but sporozoites did not resemble primate parasites. Nine sporozoite inoculations into monkeys and one in man resulted in no patent infection.
A. separatus	Quite susceptible.
A. umbrosus	Moderately susceptible since 19 per cent developed

	oocysts, but no further studies on development of sporozoites were made.
A. balabacensis introlatus	This mosquito is naturally found infected with the sporozoites. Positive salivary glands were inoculated into both man and monkey. Only one monkey became infected with *P. cynomolgi,* but none of the human volunteers demonstrated any parasites. Low level of susceptibility to *P.c. bastianellii.*
A. hackeri	*A. hackeri* is a proven natural vector of several species of monkey malaria, including *P. cynomolgi, P. fieldi, P. knowlesi, P. inui* and *P. coatney*; but very strong evidence that *A. hackeri* is not involved in the transmission of human malaria in Malaysia.
A. leucosphyrus	Susceptible.
A. pujutensis	Natural infections have been found, but laboratory infections not obtained.
A. aconitus	Susceptible to laboratory infections.
A. kochi	Susceptible to infection and transmission successfully accomplished in the laboratory.
A. maculatus	Very susceptible to *P.c. bastianelli.* 76 per cent of 450 mosquitoes became infected and transmission was successfully accomplished in the laboratory.
A. philippinensis	Quite susceptible to infection and transmission in the laboratory obtained.
A. subpictus	Perhaps not susceptible, but adequate data not available.
A. sundaicus	Very susceptible with laboratory infection of 71 per cent. Transmission successfully accomplished.
A. tessellatus	Data inadequate.
A. vagus	24 per cent became infected, but sporozoites are not known to develop.

From the above summary of the very detailed observations made by Warren *et al.* (*loc. cit.*), it seems that a majority of anophelines of Malaysia, many of which also occur in India, show a moderate level of susceptibility to *Plasmodium cynomolgi bastianellii.* But obviously there are differences in susceptibilities. The study has emphasised the need for caution in interpreting not only the finding of infections in nature but also the results of laboratory infection experiments in terms of their relevance to the field conditions.

Rodent Malaria

Though none of the Indian anophelines has been incriminated or suspected to have the capacity to transmit rodent malaria, it is of interest to note that *Anopheles*

dureni, a tree living mosquito of Africa (Katanga), has been infected with *Plasmodium berghei*. In laboratory studies several species have been infected, such as *A. quadrimaculatus* and *A. aztecus*.

Peters (1970), in his exhaustive review of chemotherapy of malaria, has pointed out that there is some evidence that certain species of anophelines in the laboratory show that they are more susceptible to the pyremethemine resistant strains of *Plasmodium berghei*. These observations, if confirmed with human malaria plasmodia, would need re-evaluation of the importance of vectors of malaria in regions where drug resistance has become established, such as *A. balabacensis* in the eastern parts of India.

Malaria of other animals

Mammals : It is now known that many of the natural infections with plasmodia found in species like *A. umbrosus, A. roperi* and others in Malaysia are not likely to be those of human malaria, but of mouse-deer malaria, *Plasmodium traguli* (Wharton *et al.*, 1963). Mouse-deer is a ruminant of the family Tragulidae, also known as Cervantines.

The role of any Indian anopheline in relation to malaria of other animals such as squirrels, reptiles or amphibians is unknown.

Bird Malaria : There is no indication that anophelines have any significant role in the transmission of bird malaria. They are generally refractory to avian plasmodia. Russell and Mohan (1942) failed to infect *A. stephensi* with *P. gallinaceum*. However, recently Gajanana *et al.* (1979), working on experimental infections in Pondicherry, have found *P. gallinaceum* infecting *A. stephensi* at an average of 10.7 per cent (range 0-28 per cent); while in a simultaneous study, *Aedes aegypti* (the natural vector) was infected at an average of 83 per cent (range 54-100 per cent). The numbers of mosquitoes tested were *Aedes aegypti* 630 and *Anopheles stephensi* 670.

Though anophelines have generally been regarded as refractory to bird malaria parasite, it is known that *P. relictum* can infect several species of anophelines. Garnham (1966), quoting the work of Hunninen (1951), mentions that under certain laboratory conditions, infections were obtained in *A. albimanus* (80 per cent), *A. freeborni* (54 per cent), *A. quadrimaculatus* (35 per cent), and *A. crucians* (33 per cent). But he stated that development of the parasite in the *Anopheles* takes longer and sporozoites are scantier.

The natural finding of what appeared to be the sporozoites of avian origin by Bhatt (1949) in *A. turkhudi* in Nasik District of Maharashtra needs to be mentioned. The sporozoites had lengths similar to those of bird malaria parasites rather than those of human sporozoites.

Filariasis

Anophelines are known to be vectors of human filariasis, though poor ones and not to the same extent as culicines, particularly *Culex fatigans* for *Wucheraria bancrofti* and *Mansonia* species for *Brugia malayi*. They also take part in the transmission of animal filariasis.

In India, according to Das (1976), natural and experimental infections of the periodic *W. bancrofti* have been detected in 17 species of mosquitoes of which five are culicines and twelve anophelines. Infective larvae have been detected in 10 species of which nine are anophelines. *A. stephensi, A. varuna, A. sundaicus, A. philippinensis,* and *A. annularis,* known malaria vectors, have also been found to be susceptible to experimental filaria infections. Other anophelines susceptible to infection are *A. pallidus, A. ramsayi, A. subpictus* and *A. nigerrimus.* However, *Culex p. fatigans* is the principal vector and anophelines play only an insignificant role, if any, in bancroftian filariasis.

Infections by *Brugia malayi* have been detected in eight species of mosquitoes of which *A. barbirostris* should be regarded as a secondary vector. It has been found naturally infected in Kerala (Iyengar, 1938a). However, *Mansonia annulifera* and *M. uniformis* are the principal vectors of this form of filariasis which occurs in India mostly in Kerala. *M. indiana* is a secondary vector.

Raghavan (1969), in tabulating the natural and experimental infections found in India, has presented the following data about anophelines:

Species	*Infection rate per cent*	*Area*	*Author/s*
A) **Natural Infections**	All *B. malayi*		
A. varuna	0.25	Kerala	Iyengar (1938)
A. subpictus	0.2	Kerala	Iyengar (1938)
A. subpictus	0.7	Calcutta	Ghosh and Hati (1966)
A. annularis	0.8	,,	,,
A. nigerrimus	1.18	,,	,,
A. barbirostris	1.17	Kerala	Iyengar (1938)
B) **Experimental Infections**	*W. bancrofti*		
A. barbirostris	40.0		
A. nigerrimus	58.5		
A. subpictus (fresh water)	72.4	Calcutta	Sunder Rao and Iyengar (1932)
A. subpictus (saline water)	76.2	,,	,,
A. sundaicus (=ludlowi)	69.8	,,	,,
A. stephensi	92.3	,,	,,
A. annularis	70.0	,,	,,
A. jamesii	40.0	,,	,,
A. varuna	84.2	,,	,,
A. pallidus	66.6	,,	,,
A. philippinensis	100.0	,,	,,
A. stephensi	86.7	Madras	Unpublished, NICD.
A. vagus	0.2	Kerala	Iyengar (1938)
A. subpictus	0.1	,,	,,
A. varuna	12.5	,,	,,

These figures show that given suitable conditions, both types of human filaria parasites can infect anophelines. But the scarcity of infections in nature indicates only an insignificant vectorial status except in purely local situations. Lahiri *et al.* (1972) have recorded the development of *Brugia ceylonensis* in *A. stephensi*. It is, however, well known that *A. stephensi* is generally refractory to infections by *B. malayi* (Krishnan, 1961) though it is susceptible to *W. bancrofti*.

In other countries of the oriental region, several anophelines have been reported to be significant vectors of human filariasis, particularly *A. nigerrimus* and *A. barbirostris*. In Malaysia, a sub-periodic form of *B. malayi* is transmitted chiefly by forest species of *Mansonia* and it develops poorly in anophelines. The periodic form of *B. malayi* is mainly transmitted by anophelines of the *barbirostris* group and the open country species of *Mansonia (indiana, uniformis* and *annulifera)*. However anophelines of the subgenus *Cellia* are generally not susceptible to either form of *B. malayi*. The possibility of some of the predominantly man-biting species of anophelines becoming vectors of filariasis in nature cannot be overruled.

The validity of the many earlier findings of natural filaria infections has been questioned. It has now been suggested that many of the earlier reports could have been due to animal filaria parasites. There are several references to natural and experimental infections of anophelines by animal filaria parasites. Raghavan (1969) in his review of the animal parasites in India, has mentioned (in his Annexure IV) that *A. stephensi* is an experimental vector of an undetermined bird filaria parasite. In Southeast Asia several records (see Harrison and Scanlon, 1975) of animal filaria infections, particularly of *Seteria*, in anophelines have been mentioned.

In summary, it can be stated that in India anophelines are generally not vectors of human filariasis, *W. bancrofti* and *B. malayi*, though experimentally they can be infected. They are more susceptible to *B. malayi* than to *W. bancrofti*. However, in Malaysia *A. barbirostris* is a proven vector and *A. nigerrimus*, a possible one.

Anopheles as Vectors of Viruses

A review of the number of viruses isolated in nature or experimentally infected in the laboratories in anophelines indicates that all over the world 42 arboviruses are involved. Many of these are accidental infections. Finding of a natural infection does not necessarily mean that the mosquito species can actually transmit the virus in nature. Its successful experimental infection and transmission in the laboratory should be taken to indicate that only such transmission could take place under favourable natural conditions. The factors which make a mosquito a vector of arbovirus in nature are quite complex (Ramachandra Rao, 1975). Not only laboratory infections, but also epidemiological evidence is necessary.

A well known arthropod-borne virus which has been the cause of large epidemics in Africa is Onyong-nyong virus transmitted by *A. funestus* and *A. gambiae* (Theiler and Downs, 1973). An epidemic affecting millions of persons occurred in the 1950's in Uganda, Tanzania, Kenya and Nyasaland. It is a dengue-like disease closely similar to chikungunya, both of which occur in India.

In India, the following viruses have been isolated or experimentally infected in anophelines:

Name of virus	*Species*
Arkonam	*A. subpictus, A. nigerrimus* (natural infection)
Batai	*A. subpictus, A. tessellatus* (natural isolation)
Calavo	*A. stephensi* (experimental)
Chandipura	*A. stephensi* (experimental)
Japanese Encephalitis	*A. barbirostris, A. nigerrimus* (natural infection).
	A. tessellatus (experimental infection only)
West Nile	*A. subpictus* (experimental)

In addition, a few experimental infections of other non-Indian viruses in Indian species of anophelines such as *A. stephensi* and *A. philippinensis* have been obtained in other countries.

Table 8 gives a list of anopheline mosquitoes from which arboviruses have been isolated in nature in India.

Table 8. Arboviruses isolated from Anophelines in India

Species	*Virus isolated*	*Locality where isolated*	*Cited by*
A. barbirostris	Japanese encephalitis	Asansol (West Bengal)	Chakravarty *et al.* (1975)
	Batai	Brahmanpalli Chittoor District (Andhra Pradesh)	Singh and Pavri (1966), NIV (1979).
A. "hyrcanus" group	Japanese encephalitis	Asansol and Bankura (West Bengal)	Chakravarty *et al.* (1975) and Banerjee *et al.* (1975), NIV (1979).
	Arkonam	North Arcot District (Tamil Nadu)	NIV unpublished data (1970).
A. subpictus	Arkonam	North Arcot District	Dandawate *et al.* (1969).
	Batai	Manjri, Pune District (Maharashtra)	Singh and Pavri (1966).
A. tessellatus	Batai	Manjri, Pune District (Maharashtra)	Singh and Pavri (1966), Theiler and Downs (1973).

NIV = National Institute of Virology, Pune.

The finding of natural infections of Japanese encephalitis virus in Bengal (Asansol and Bankura) in the recent epidemics between 1973 and 1978 is of great interest. The species involved were *A. barbirostris* and a member of the *hyrcanus* group probably of *A. nigerrimus*. These findings have been followed by experimental infections and successful transmission in the laboratory by *A. tessellatus*. The role of anophelines in the epidemiology of arboviruses in India, therefore, assumes a

great importance and needs immediate detailed studies. It may be noted that *Culex tritaeniorhynchus* was hitherto considered to be the major vector of Japanese encephalitis virus. Many isolations have been made in South India, from this species supported by laboratory transmissions. Pig was considered the main zoonotic reservoir, but recent studies tend to add certain ardeid birds as additional hosts (NIV, 1979).

Very recently natural infections of J.E. virus are reported to have been made from *Anopheles subpictus* in Karnataka State (NIV unpublished data, 1980).

7

Vector Density Factor in Malaria Epidemiology: Some General Considerations

Density of the vector is among the most important factors in the epidemiology of malaria. It determines the degree of contact between man and the vector and therefore the intensity of malaria transmission.

Even if a species has all the characteristics which can make it a very efficient vector—being highly susceptible to infection, long-lived and biting man by preference—it can have no place in transmission in a locality if it does not occur in adequate numbers. Each female mosquito has to live for at least 10 to 12 days before it can transmit the infection to a healthy individual. It is now known that the proportion of the mosquito population which lives upto the dangerous age is rather low; with most species, it is less than 5 or 10 per cent. Further, the opportunities for feeding initially on an *infected* individual host are by themselves small, and the chances of the same mosquito biting a second *uninfected* individual after a lapse of 10-12 days are still less. If so much malaria still exists, it is due to the high densities of the vector.

Even simple arithmetic, without the help of any sophisticated formula, would show the importance of numbers. If 10 per cent of the vector females bite man on an average (AI=10per cent) and only 5 per cent live longer than 10 days (as with *A. culicifacies* under favourable conditions), the chances of an individual mosquito to take a first blood meal on man and another on another man 10 days later are: 1/10 x 1/20 x 1/10=1/2000, i.e., only one mosquito out of 2,000 individuals does so. When it is further noted that only a fraction of the total females are likely actually to feed on gametocyte carriers and become infected, the importance of sheer numbers can be appreciated. On the other hand, with species which bite man by preference, such as *A. fluviatilis* (AI=80 per cent) of which 20 per cent live longer than 10 days the initial numbers required will be much less (8/10 x 1/5 x 8/10=64/500). 64 out of 500 females can bite man the second time after a lapse of 10 days. Commencing with the same 2000 individuals, 256 or about 12.8 per cent can take part in transmission. Here again not all females become infected. But because of the man-biting habits the chances of a mosquito becoming infected are very much higher than in the former case. Therefore, it would be clear that wide variations can exist in the numerical requirements of the vectors depending on the bionomics of the species. Ronald Ross, as far back as 1911, had appreciated the importance of this factor in malaria epidemiology.

Density may be theoretically defined as the number of vectors present in a unit

area, say one square kilometre, at a given time. If the number could be determined and the number of humans present in the area known, it is possible to calculate the number of vectors per persons. However, the determination of such a figure is neither easy nor practicable because of factors like infiltration, dispersal and the availability of animals which attract a proportion of the vectors, apart from lack of proper technology.

The density, or better the number, of vectors at a given moment is itself influenced by many factors. Some of these are:

(a) The availability and extent of suitable breeding places.
(b) The intensity of breeding and output of adults which are influenced by physical and biological factors.
(c) Longevity of the adults influenced by atmospheric conditions and the effect of parasites and predators.
(d) The degree of infiltration and exodus as influenced by the scatter of breeding places and the concentration or scatter of the hosts to feed.
(e) The scatter or concentration of suitable resting places and so on.

Absolute and relative densities

While there are techniques for estimating the total numbers actually present, viz. absolute densities (see Service, 1976) within a reasonable degree of precision, they are rarely suitable for a routine malaria entomologist to adopt. Reliance has to be placed on simpler and more direct methods, which give expression to "relative densities" rather than to absolute densities. Relative densities are useful to compare the prevalence of a vector in the same area between seasons or between years, and to compare them between two different places at the same time. They are actually "indices" but are of much practical utility.

Malariologists have been expressing relative densities in several ways.

(a) Average number of vectors collected in human or animal dwellings (or even in outdoor shelters) by hand collection done by standard methods by searches over fixed periods of time—the *per manhour* figure.
(b) Average numbers collected in dwellings, to *depletion*, either by hand collections or by use of knock-down insecticidal sprays such as pyrethrum sprays.
(c) Average numbers of adults collected actually biting man or selected animals at night; either throughout the night or at selected periods of the night.
(d) Average numbers collected by the use of
    i) Traps, special huts, or nets, using man or animal as bait.
    ii) Traps using light or CO_2 as attractions.
    iii) Artificial shelter created outdoors.

Of the methods mentioned above, the determination of "per man hour" figure has been used most extensively, not only to study fluctuations in the abundance of mosquitoes but also to assess the success or failure of control measures. Usually a

sufficient number of dwellings are searched by trained insect collectors, each for a period of 15 or 30 minutes, by use of standard suction tubes and flash-lights, and the number of adult mosquitoes is recorded. The total number collected in all the dwellings is then averaged and reduced to a figure, "per manhours". Sometimes when dealing with species which are scarce, the figure for "10 manhours". is employed. This method works reasonably well when the acutal numbers in dwellings are neither too large nor too small. In the fomer case, the collection has a maximum limit and the total collected does not truly reflect the actual number; in the latter case, much time is wasted by searches over empty surfaces and the number actually collected is incommensurate with the effort made. It is also to some extent influenced by the "efficiency" of the insect collectors which however can be evened out by alternating them on different occasions.

However, the "per manhour" figure does not give any indication of the actual number biting man per day, which is perhaps the most important entomological parameter affecting malaria transmission. There is no method of correlating the p.m.h. figures with the actual numbers biting man. Further, in case of species which have a proportion resting out of doors during day-time, the indoor collections reflect only a partial segment of the total population. Nevertheless, the per man-hour figure has been a practical index of great utility in routine malariology.

There are several methods of "estimating" the total adult vector population by application of appropriate sampling techniques. Many of them are described by Service in *Mosquito Ecology—Field Sampling Methods* (1976).

Perhaps the most useful of them is the "mark-release-recapture" method. In this method a known number of adult mosquitoes of a known age is marked by suitable methods, such as by coloured powders, fluorescent dyes, P^{32} tagging, genetic markets, etc., and released into the environment. On subsequent days, all mosquitoes resting in randomly selected dwellings are searched for. The proportion of the marked ones to the unmarked ones is found out and by taking note of factors such as survival rates and dispersal etc. the total number of mosquitoes in the population is estimated on the basis of the proportions of the marked ones to the unmarked ones. The method has been used extensively in entomological studies with many kinds of insect. In India the method has been used by Reuben *et al.* (1973) with *Aedes aegypti*, and by Yasuno and Rajagopalan (1973) with *Culex fatigans* both in Delhi. The method has not been used with anophelines in India, though some studies have been made abroad. The method has received wide critical attention and several methods of analysis have been developed by workers such as Lincoln, Baily, Fisher and Ford, Sheppard and others.

Both relative and absolute densities can be used in the measurements of the dynamics of malaria transmission provided they are used with caution.

Numbers biting man per day

The actual parameter reflecting man-vector contact is the number of vectors biting man per day (or night). This has also been estimated in several ways and used in

mathematical models as will be referred to below. A point to keep in mind in such studies is that many vector species also bite domestic or other animals and the numbers biting man, are only a proportion of the total population. The proportion varies from place to place and season to season depending upon the availability of the animals. Methods of determining the numbers biting man are available: direct methods such as collecting the mosquitoes actually biting man or indirect ones like determining the anthropophilic index for a given place at a given time.

Density of vector and malaria endemicity

From the earliest days after Ross's discovery of the role of anophelines in malaria transmission, numerous attempts have been made to determine how best the disease could be controlled. It did not take long to recognise that it was not necessary to control all anophelines but only the species which was the vector in a particular locality or region ("Species sanitation"). Also it was not necessary to eradicate the vector species, it would be enough to reduce its numbers to low levels in order to obtain good control of the disease. The entire work of Malcolm Watson and others in the second and third decades of this century in Malaysia, Assam and other places was based on the principle of *reduction in numbers* of the vectors.

One of the practical uses of the "per manhour" method has been to estimate the "Critical density" of a vector, a concept developed by Ross (1911) in his book, "*Prevention of Malaria*", according to which it was not necessary to reduce the vector population to zero levels to obtain cessation of malaria transmission. The idea of a "critical density" level was exemplified by Barber and Rice in Macedonia, and in respect of Indian anophelines by Russell and Ramachandra Rao (1942) in their comparative studies on the densities of *A. culicifacies* in the malarious Pattukkottai Taluk and non-malarious Cauvery Delta in Thanjavur district. They used the "per manhour " figures in their study. In the former area p.m.h. figures were high throughout the irrigation season, reaching and persisting at 50 p.m.h. for many weeks. In the latter it was continuously low with a brief peak for one week only in July (see under *A. culicifacies*).

They noted that when densities were below 8 to 17 per man hour, there was no malaria transmission. However, for purposes of safety in undertaking practical malaria control they suggested "5 per manhour" as the "critical density" for the species in that area.

The idea of "critical density" was later taken up by several workers in India. Viswanathan *et al.* (1944) estimated a critical density of 4 per 10 manhour for the highly anthropophilic *A. fluviatilis* in North Kanara District. Later Jaswant Singh *et al.* (1957) discussed the subject of critical densities among Indian malaria vectors and projected the following figures for adoption in the N.M.E.P.

A. culicifacies	33.0	per 10 man-hours
A. stephensi		-do-
A. stephensi var. *mysorensis*		-do-

A. varuna		-do-
A. philippinensis	13.0	per 10 man-hours
A. sundaicus		-do-
A. annularis, *A. sundaicus*, *A. fluviatilis*, *A. varuna*	13.0	per 10 man-hours together
A. culicifacies, *A. fluviatilis*		-do-
A. philippinensis, *A. sundaicus*		-do-

There is no need for any deep study to understand that "critical densities" can vary considerably from place to place. The three main components whose quantitative changes contribute to variation in malaria prevalence are:

A- numbers of the vectors biting man per day.

B- numbers of gametocyte carriers in the community at a given time.

C- numbers of non-immune individuals in the community at a given time.

A graphic description of this well known principle has been given by Russell *et al.* (1963), and recently by Gabaldon (1978), and has also been re-emphasised by Ramachandra Rao (1979).

It would be a mistake to expect a uniform set of 'critical densities' for any vector species all over India. It is essential for such a density to be estimated for each vector and region after careful study of all epidemiological factors.

Density in relation to use of insecticides

While 'critical density' does have epidemiological relevance and also a practical value as an indication of a safe target to aim at when anti-larval measures are being undertaken, it is questionable whether it has the same utility when anti-adult measures like pyrethrum space sprays or DDT residual sprays are adopted.

Pyrethrum sprays kill mosquitoes which are actually present in the houses and cattle sheds at the time of spraying. In the process, they kill infected mosquitoes both those which had already reached the infective stage and those which had just taken infective blood-meals. Malaria control is achieved by the destruction of the infected mosquitoes. As in some species there is a considerable degree of outdoor resting, pyrethrum spraying once a week can kill only a proportion of the total population making a negligible impact on the total population. Nevertheless it can effectively control malaria transmission. In fact, it was very useful against *A. culicifacies* because of the zoophily and low infection rates of the species. It was, however, not a success against *A. fluviatilis* because the latter besides being highly anthropophilic, had very high infection rates and a sizeable proportion of it rested outdoors. Viswanathan *et al.* (1944) suggested a 4-days a week spraying programme, while Senior White (1945) wondered whether even a 6-days a week pro-

gramme would be effective. Once a week pyrethrum sprays make little impact on the total populations. Pyrethrum sprays act only by killing the infected specimens and reducing their numbers.

DDT and other residual insecticides, on the other hand, have quite a different type of action. They kill the mosquitoes actually resting inside the houses and cattle sheds, not only on the day of spraying but also during subsequent days for several weeks. Some may escape by not getting an adequate contact with the insecticide the first time, but the residual deposits stay on the sprayed surfaces inside the premises for several weeks and the vectors have many more chances of coming into the premises again for feeding or for resting and getting further toxic contacts with the insecticides and consequently, of dying. Everyday sizable proportions of the populations are killed. Residual insecticides have the effect of reducing the average length of life of the susceptible vectors. Because of the mortality of large proportions of the vector population every day (or night), very few, if any, live upto the dangerous age of 10 to 12 days. Studies by the use of Detinova's technique of determining age have shown that in recently sprayed villages specimens which have oviposited more than once are rare and frequently most members of the vector female population are nulliparous. In such circumstances the question arises whether the 'critical density' of a vector has any significance.

In a normal situation DDT spraying, if the vector is susceptible, leads to an immediate fall in indoor densities, but the densities gradually build up. In such a situation one has to see the reasons for the build-up of adult mosquito populations inside sprayed structures. It could be due to one of the following:

1. Mosquitoes are becoming resistant to the insecticides and, therefore, are not dying.
2. The residual deposits of the insecticide on the sprayed surfaces are gradually disappearing and the adults find surfaces to rest on inside the premises without insecticidal deposits.
3. A sudden unusual increase in breeding has occurred leading to massive output of adults.

Are the same levels of critical densities applicable in all such cases? This is a moot question. If the average length of life of the population which has reappeared is about the same as in a normal population, the predetermined "critical densities" or "tolerated densities" (a useful term coined by the N.M.E.P. workers) would be applicable. However, if the insecticide has still got some effect and the average length of life is less than in a normal untreated population, densities much higher than the normal can be accepted. Measurement of the age of the vector females are of very great importance and may save a lot of unavoidable effort and expenditure on insecticides.

These are not mere theoretical considerations. It would be prudent, as has been done by the N.M.E.P. of India to establish certain 'critical', or better 'tolerated' densities, the build-up of which would be an indication that the next spray is needed. The whole question of 'critical density', the existence of which has been established, needs a thorough study under different types of situations.

Of course with species which predominantly or exclusively rest outdoors, such as *A. balabacensis*, or even *A. philippinensis* in recent times, there can be no critical densities based on per man-hour figures based on indoor collections. Other types of expression would have to be adopted.

Vector/man contact

One of the trickiest questions is how to measure and express densities of anophelines in relation to man. The per man-hour figure which is being used is an artificial index though extremely useful in routine epidemiology and control. It is difficult to estimate the true or absolute density from the per man-hour figures. Apart from not taking into consideration the large numbers which may be resting outdoors, there are considerable differences in the per man-hour figures in dwellings even between forenoons and afternoons (Viswanathan *et al.*, 1950; Wattal, 1962).

Further, mosquito adult populations are not uniformly distributed in any given area. They tend to become concentrated in houses or cattle sheds or natural shelters. Even day time resting places do not have any uniformity in size, scatter or attractiveness to the imagines. Therefore, estimates of anophelines present in an area is beset with many problems both theoretical and practical. The p.m.h. figure is not refined enough for precise epidemiological studies.

If a correct estimate of the number of vectors biting man per night, on the average, can be determined, it would be possible to assess the degree of malaria transmission at a given place at a given time. Pending development of accurate techniques, malariologists would have to make rough estimates using a combination of several available techniques.

Mathematical models for malaria transmission

Some useful mathematical models for determining the degree of transmission have been developed and put into practice.

The mathematical aspects of malaria transmission based on the main factors have received attention from many authors. The most widely known is the one evolved by Macdonald (1957) defining a reproduction rate of malaria.

Reproduction rate

After reviewing the work of many authors, including the basic studies on such aspects as biting habits, longevity, densities, etc. done in India and Africa, Macdonald evolved a formula to determine what is known as the reproduction rate.

The proportions of mosquitoes surviving for n days is p^n and their subsequent expectation of life will be $\frac{p^n}{-\log_e p}$

During this time they will bite a times each day and the proportion b of these bites

will be infective. Therefore,

$$Z = \left[1 - \frac{ax}{ax - \log_e p}\right] \frac{ma^2 bp^n}{-r \log_e p}$$

where Z = reproduction rate;

m = anopheline density in relation to man;

a = average number of men bitten by a single mosquito in one day;

b = proportion of those anophelines with sporozoites which are actually infective;

p = probability of the survival of a mosquito through one whole day;

r = proportion of people who have received one infective inoculum only, who revert to the uninfected state in one day;

n = time taken for the completion of extrinsic incubation period cycle; and

x = proportion of people affected.

When x approaches zero, the limit of the rate is $Z_0 = \frac{ma^2 bp^n}{-r \log_e p}$ which is termed the basic reproduction rate. The value of the reproduction rate not only depends on the degree of immunity in the human population, but also on mosquito densities. The formula has been further elaborated and those interested should read Macdonald's articles in original.

If the reproduction rate is one, malaria transmission continues on the same scale. If the rate is more than one, the incidence of malaria will increase. If the rate is less than one, malaria gradually diminishes and finally disappears.

The formula has been tested in several types of situations and with several vectors and has largely been found valid. Only recently there has been some criticism on the 'r' values, i.e., the proportion of affected people who have received one infective inoculation only and who revert to uninfected state in one day. The importance of super-infection has been re-assessed and to that extent Macdonald's formula needs some modification. (Fine, 1975).

As a result of the theoretical consequences of the formula, Macdonald has shown how epidemics affecting 50 per cent of the population can occur in seven months even if the reproduction rate is as low as 5, and only 3 to 4 months if the rate is 20. It is the mosquito factor which is liable to gross and rapid changes and, therefore,

has a profound influence on the reproduction rate. Taken from Macdonald,the figures in Table 9 show the importance of the reproduction rate.

Table 9. Time taken in days (approx.) for 50 per cent of the human population to become infected at different reproduction rates, with an initial parasite rate of 0.1 per cent

Reproduction rate	*vivax*	*falciparum*
5	215	over 300
10	155	220
20	110	160
50	80	120
100	65	100

There is difference between the time taken for epidemics with *P. vivax* and *P. falciparum* because *vivax* has a shorter incubation interval and, therefore, the increase in number of cases is more rapid.

As nothing biological in nature is static or constant, one may expect many types of variation in the malaria situation. It is, however, necessary to realise that the mosquito factor, as finally computed as the inoculation rate, is very important in malaria epidemiology. All inoculations, however, are not necessarily successful due either to small numbers of sporozoites or the immunity of the hosts.

Macdonald's numerous articles on the subject culminating in his book, *Epidemiology and Control of Malaria* (1957), should be read by all interested in the dynamics of malaria transmission.

Ray (1966)-attempted to simplify the factors involved in malaria transmission. The transmission potentiality (*TP*) of an area could be determined by a simple formula:

$$TP = CD \times L \times APL$$

or

$$TP = R \times APL$$

CD = Critical density of the vector.

L = Longevity of the vector.

APL = Annual parasite load, *i.e.*, number of people with infection.

R = Receptivity.

Among the entomological parameters Ray discussed the concept of 'critical density'. He stated:

> "Having taken the various factors into consideration, including certain common operational shortcomings in the application of residual insecticides, the average expectation of life of the vector for the purpose of present discussion may be assumed to be 3 days (average) in attack phase area. Similarly, the reduction in the density be assumed to be one-tenth of the critical density in respect of *A. fluviatilis* and *A. minimus* as recorded prior to

the commencement of insecticide spray operation and to about half in respect of *A. culicifacies.*"

The important thing in DDT spraying is the lessening of the average length of life as Pampana (1962) has shown. A critical density may not be quite as applicable during a programme using residual insecticides as in normal epidemiology; but as a practical yardstick for routine operational use, it is acceptable.

Two other entomological expressions which have been considered are 'inoculation rate' and 'vectorial capacity'.

Inoculation rate

Inoculation rate can be summarized by the expression:

$$h = m\ a\ b\ s$$

where

h = number of positive bites received per person in one night;

m = anopheline density relative to man;

a = the man biting habit of the anopheline species;

s = the sporozoite rate of the biting populations; and

b = the proportion of anophelines with actually infective sporozoites.

This involves the determination of the sporozoite rates. The estimation of the inoculation rate is not considered to be of such practical value by itself because the requisite end result can be obtained by parasitological surveys. But as a confirmatory complement to parasite surveys, it can be of value (*WHO Manual on Practical Entomology in Malaria,* 1975, Part I).

Vectorial capacity

The concept of 'vectorial capacity' (Garrett Jones, 1964) is the same "as the basic reproduction rate of malaria but expressed on a daily basis to determine the force of infection in a particular epidemiological situation." (WHO, 1975, *Manual of Practical Entomology*).

Vectorial capacity $C = \dfrac{ma^2p^n}{-\log_e p}$

p = probability of survival through one day;

m = anopheline density in relation to man;

n = time taken for extrinsic incubation cycle;

a = average number of human feeds per mosquito per day; and

e = base of natural logarithms.

In other words, vectorial capacity is "the number of potentially infective contacts

an individual person makes, through the vector population per unit time". Critical vectorial capacity is the minimum value of C below which malaria cannot maintain itself at an endemic level.

The main use of the vectorial capacity concept is in attempting to monitor transmission potential in areas where no transmission is taking place, but which are both vulnerable and receptive. Comparison of vectorial capacities before and after anti-vector control measures would help to measure the effect on the vector populations and transmission.

Deitz *et al.* (1974) have tested the idea of vectorial capacity in an African savannah, in Karo State, N. Nigeria, including studies on the role of superinfections.

Batra *et al.* (1979) have estimated the vectorial capacity in *A. stephensi* in Salem Town, Tamil Nadu, with *P. vivax* infections:

Shevpet	— 0.09 to 0.43 (corresponding to biting rates of 0.08 to 1.0 per man per night).
Arasipalayam	— 0.07 to 0.50 (corresponding to biting rates of 0.2 to 1.1 per individual per night).

They said that it was difficult to determine accurately the critical vectorial capacity. If 7 days are accepted as the average duration of infectivity of human cases in Salem, the critical vectorial capacity is 0.29 or lower.

More recently Dutertre (1976) has attempted to improve the known mathematical models by taking note of factors related to the human hosts and their response to infection. The inoculation rate adjusted by him corresponds to that of Bailey (*Mathematical Theory of Epidemics*, London, 1957) in which

$$S = \frac{ma\ G_e^{-qn}}{(a\ G/N)+q}$$

where G = ratio of gametocyte carriers with regard to the total population N,

ma = the man biting rate of *Anopheles;*

q = their daily mortality rate; and

n = time taken for the extrinsic incubation.

The above brief presentation of rather complex mathematical models has been made only to highlight the need for quantifying various parameters in malaria epidemiology and applying them to understand the dynamics of malaria situation at any given place, both for epidemiological purposes and also as a practical tool for routine operational use in control programmes. The determination of the degree of man-vector contact is the most crucial requisite in such applications and Indian entomologists have an excellent opportunity to study the subject.

In view of the lessened efficiency of synthetic insecticides applied as residual sprays, greater reliance may have to be placed in future on anti-larval measures and

on attempts to reduce vector densities in relation to man. The application of the idea of 'critical densities' again assumes great importance. As there is little immediate prospect of eradicating any of the important vectors, grounds of economy alone would justify just reducing the densities to levels below the 'critical density' of the vector.

Attempts to understand and express malaria epidemiology in mathematical terms have been made by N.R. Rao and co-workers (Rao, N.R., *et al.*, 1974, 1975, 1976 and 1977). They have found the critical levels of mosquitoes to man required for malaria transmission to be higher than those obtained by Macdonald possibly explaining the phenomenon of anophelism without malaria.

Larva density

The measurements of larva densities are equally important in malariology. Unfortunately the subject has not received the attention it deserves. It is the larva density which determines the adult density. However, the measurements of larva densities are of a complex nature because of the variety of breeding places, each differing from the other in physical and biological characteristics.

Measurement of larva densities is important for several reasons. (1) Seasonal and annual fluctuations in the actual intensity of breeding in any place can be watched. (2) The effect of anti-larval measures can be assessed. (3) Differences between types of breeding places can be determined so that attention may be devoted to the most important ones, i.e. those which contribute substantially to the total adult population.

The last item is extremely important not only for epidemiological studies but also for organising control operations. The ecologically ideal breeding places may not necessarily be the epidemiologically important sources, or vice versa. If the true importance of any type of breeding place can be determined, either greater attention can be given to it or it may be ignored as inconsequential.

The well-known example of *A. fluviatilis* is relevant in this connection. Though, ecologically, streams and channels with a slight flow are its most preferred places, it also breeds in a low degree in certain types of rice fields. The total output of adults which contribute to the malaria situation is however higher from ricefields because of their vast surface areas compared to those of channels and streams. Of course, this occurs only in seasons of rice cultivation. In other seasons streams and channels are the most important breeding places. Similarly in the case of *A. culicifacies* though river bed pools have the most intense breeding, they are epidemiologically insignificant when compared with the extensive breeding which takes place in ground pools, irrigation channels, borrow pits, etc. Only in dry seasons, or under drought conditions, when only river bed pools occur, are the latter really important, as happens in many river-side villages in the Deccan plateau (Sri Lanka epidemic of 1935 is a good example).

Therefore, it is essential to employ reliable methods of measuring and estimating densities of larvae in different types of habitats. One of the most useful methods is

to sample the breeding places by use of standard dippers. A fixed number of dips is made by skimming the surface of water at random and the average number of larvae per dip is estimated. This can be useful in most types of medium or large sized water surfaces—but not in small pools as turf pools, hoof marks, seepages, etc. In such places larvae are collected with the help of spoons, ladles and even directly with pipettes.

A dipper of standard size is necessary. There are many variations but the one illustrated by Russell *et al.* (1963) in their *Practical Malariology* (page 284) is among the most satisfactory for the majority of situations. No single method is, however, applicable to all types of breeding places. Since ordinary dippers fail to be useful in certain types of breeding places, alternative methods will have to be adopted.

In a detailed analysis of over 1,98,430 larvae of all species (out of which *A. culicifacies* numbered 68,259) collected over a period of 33 months (1937-38) in Puttukkottai Taluk of Tamil Nadu, by using the standard dipper, in collections totalling over 6,000. Russell, Ramachandra Rao and Putnam (1945) studied the comparative utility of the number of dips, approximate surface area searched and the actual total time spent in minutes. Using partial correlation techniques, they concluded:

1. Good correlation exists between the logarithms of the monthly captures and either collection unit, minutes or square feet. The relationship is, therefore, exponential in character.

2. When regression equations, expressing the relationship between larvae captured and both minutes and square feet, are computed, the minutes spent dipping are found to be the determining factor. Area covered does not contribute to the number of larvae captured when time is held constant. Captures in wells during 1937 and in borrow pits throughout the period are exceptions to this rule.

3. The precise form of the equation describing the relationship differs for larvae captured from different habitats. It also changes from one year to another.

4. For routine field use, a simple ratio of larvae captured to minutes spent dipping furnishes a practical measure of density.

The present author has used this method with reasonable success in many areas. However, the method again gives an index and not an absolute density. Though the use of "larvae collected per unit time" is a valuable method, it has rarely been used by others in routine malariology.

There are also more refined methods of estimating the size of populations. One of these is to enclose areas of the breeding places in bottomless tins of several sizes and count all the larvae within them. By determining the number per square foot, a reasonable estimate can be made of the total breeding in certain types of shallow breeding places such as ricefields, tank margins, etc. But the method is not applica-

ble to breeding places where the larvae cling to the edges as in streams, channels, wells, etc. The method has a very limited use only for very special ecological studies and is inapplicable in general surveys. (See Figure Nos. 99 and 100, page 288 in Russell *et al., Practical Malariology,* 1963). This method had been used earlier by Cambournac, Rice and others in Portugal.

Service (1976), in his monograph on sampling techniques in mosquito ecology, has given an exhaustive review of the methods which have been and can be used.

Additional information on larval numerical ecology can be obtained by determining daily survival rates, the rates of pupation and the rate of output for adults. The last can be estimated by setting up "output-nets" over breeding places and collecting all adults emerging from a unit area. Many kinds of trap nets have been used all over the world, but in India reference may be made to the work of Russell and Ramanatha Rao (1940) and Muirhead Thomson (1942). A good type of net suitable for edges of tanks used by the present author is illustrated in Figure No. 100 in Russell *et al.* (1963).

The relationship of the observations on the intensity of breeding in different types of habitats to the actual density of the adults produced is an extremely complex subject and will not be discussed here. However, an experienced malariologist can gauge the relative importance of breeding places by practical judgment and also by indirect methods of controlling breeding in different types of habitat, and then evaluating the resulting reduction in the adult population.

The quantitative aspects of mosquito bionomics and ecology in relation to disease transmission are again being appreciated and a vast field of research is open to field biologists.

8

Landmarks in Control of Anophelines as a Means of Malaria Control in India

After the epoch making discoveries by Ross in India and Grassi and colleagues in Greece on the mosquito transmission of malaria, control of anophelines as a means of control of malaria became a favourite subject for study and experiment in many parts of the world. In India the very first effort in this direction proved to be a flop. Detailed studies had been initiated by a commission of the Royal Society of London to prosecute enquiries in malaria in India. The Commission felt that in North India, especially in the Punjab, conditions were favourable for operations against mosquitoes. They decided, in consultation with the military authorities, to conduct an experiment in a cantonment in North India. Mian-Mir, a cantonment near Lahore, was selected. Extensive and costly schemes of drainage and other engineering works were not envisaged. The object was merely to demonstrate the practicability of diminishing malaria by minor and inexpensive methods suggested by Ronald Ross himself in 1902. The work was started by Major James in April 1902. Breeding was sought to be controlled in canals, irrigation ponds, rainfilled pits, surface drains, shallow standing water like ponds, and miscellaneous places. The work was continued by Captain Christophers (later the famous Malariologist/Entomologist) from July to November 1903. The conclusion was that it was not possible to destroy *Anopheles* mosquitoes in the cantonment by simple and inexpensive methods. It was noticed that the malaria bearing mosquitoes could travel long distances (3/4th of a mile or more). A much more comprehensive scheme was needed. From 1904 to 1909 more intense work was done both on control and on the study on the habits of anophelines. The summary of the work has been given by Christophers (1904) and by Norton *et al.* (1910). The work was considered a failure and the need for further research and experiment was stressed. The amount of the organisation, supervision, labour and expenditure, devoted to such a task was considered greater than would be possible in any Indian town or hamlet. The methods adopted were mainly connected with the elimination of breeding places by drainage and filling. The earlier report by Christophers (1904) is of interest because many breeding places which could not be baled out were treated with kerosene oil from a watering can!

Since the very limited operation done in Mian Mir, many vigorous studies and attempts had been directed towards the control of anophelines. The subject is so extensive and the reports are so numerous that only passing references can be made to a few of the major landmarks in the progress of *Anopheles* control as a means of malaria control in India.

Control of Urban Malaria

Bombay City

In the first decade of this century, Bombay city was noted for its great unhealthiness due to malaria. Bentley (1911) has referred to the allusion frequently made to the unhealthiness of the city because of fevers from times dating as far back as 1673. In a scientific survey of blood examinations in 1903, out of 3,413 cases of fever detected among the City Police, 2,542 persons had malaria parasites in their blood. Bentley (1911) made an interesting report on the causes of malaria and recommended certain measures. He determined *A. stephensi* as the vector of which the permanent breeding places were wells, cisterns, fountains, and garden and other tanks. He considered malaria control in Bombay to be much easier than in many other areas of the country. The measures he recommended for control of the vector were prohibition of construction of new wells, existing wells to be registered and licenced, unwanted wells to be filled up or permanently closed, with a pump attached (if desired), fixing up of trap doors, stocking wells with fish and cisterns to be rendered mosquito proof, etc. House owners who refused to comply were to be made liable to penalty. Work was organised on these lines but with varying degrees of success. Bentley should, indeed, be regarded as among the pioneer malariologists in the country. As usual complacence set in and the anti-malaria staff was drastically cut. Malaria outbreaks occurred periodically. It was considered that the outbreaks were due to the diffusion of infected mosquitoes from houses in the Fort area into the dock area. In certain areas aggregation of labour from various parts of the country added to the problem. Captain B.S. Chalam was put in charge of anti-malaria operations, and he succeeded, according to Covell, in keeping the disease in the Worli and Fort areas under complete control. Then Major Covell (1928) himself made an intense study of the problem and made recommendations for a thorough reorganisation of the work including the need for a well paid malaria staff*. The recommendations which were in considerable detail including penal provisions under the Municipal Act are given in his book *Malaria in Bombay*. The revised work resulted in practically complete elimination of malaria in old Bombay city except for introduced cases from the highly malarious neighbouring areas of the old Bombay State. The work in Bombay city is one of the shining examples of the success of the anti-mosquito work in urban areas anywhere in the world. The anti-larval measures were continued on a firm basis till the advent of DDT and even subsequently.

*How persistent Covell was on the need for a permanent staff to deal with malaria in India can be seen from the fact that in 1940-41 he strongly urged the Bombay Government to establish a Permanent Malaria Organisation. Such an organisation did indeed come into existence in April 1942 being permanent from the outset. He insisted that such organisations should have permanent full-time qualified entomologists, a thought not well appreciated till much later by other State Governments. However, it was a human factor that precipitated the expeditious action. The Governor of the state had to fall ill from malaria after a brief hunting trip to the forests of North Kanara District!

Delhi Urban area

Delhi city, was notorious for the prevalence of malaria. The malaria situation among the troops was so intense in 1904 that the bulk of the garrison had to be removed to the Ridge about 2 miles to north and west during the worst months. However, it was not until 1936 that well planned anti-malaria operations for the Delhi urban area were seriously undertaken. It was by then well known that the vector in Delhi was mainly *A. culicifacies*, with *A. stephensi* playing a minor role. Delhi was also liable to periodic regional epidemics characteristic of the Punjab.

Covell (1934) has given a concise account of the malaria control problem in Delhi and the various measures which were undertaken. Though the work was well conceived, its execution left much to be desired. He made stringent recommendations for anti-larval measures in breeding places like wells, cisterns, borrow pits and excavations, domestic breeding places, drains and nullahs and ornamental waters up to a distance half a mile beyond the boundaries of the old and new city.

The major anti-malaria activities in Delhi, prior to the use of DDT, were: (*a*) filling and draining of water collections wherever possible; (*b*) use of Paris green and oil in a systematic manner in all breeding places not amenable to filling or draining; (*c*) straightening and lining of all nullahs; and (*d*) introduction of *Gambusia* fish in wells and other suitable places such as tanks. The work was integrated with the control of *C. fatigans*, the nuisance mosquito. By these methods the incidence of malaria was greatly reduced. However, these measures did not affect the malaria situation in the rural areas of the Delhi State. In this connection, it would be interesting to note that the selection of the site for New Delhi was made very carefully in the early twenties and the present site was selected because of its higher elevation and the distance from the Jamuna river.

After DDT became available, it was used in the peripheral riverine semi-urban areas in addition to the anti-larval work. Later, use of DDT was extended to all the villages of the Delhi State. While initially the results were very satisfactory, there has been a gradual slackening of anti-larval measures in recent years in the city mainly because of indifference as a result of reliance on DDT only.

Jaswant Singh (1962) has stated that 'the chief malaria problem in Delhi is due to the excessive canal irrigation, coupled with interference of natural drainage by railway, road and canal embankments and annual flooding of the Bela by the rise in water levels in the river Jamuna, resulting in heading back of water in the various storm water drainage canals and the formation of prolific breeding places as the flood recedes. The presence of a number of excavations throughout the area in the form of railway and roadside borrow pits, pits in the brick fields and stone quarries form additional hazards'.

The breeding of *A. stephensi* in wells added to the problem.

There has been a progressive deterioration of the malaria situation in Delhi due to causes such as development of resistance to insecticides in the vectors, inadequacy of anti-larval work directed specifically against anophelines, the rapid and phenomenal growth of the city in all directions leading to very vigorous house constructions and consequent increase in the breeding of *A. stephensi*. Dhir (1969)

reported on the outbreaks of malaria caused by *A. stephensi* found breeding in buildings under construction all over the city. Perhaps *A. stephensi* has replaced *A. culicifacies* as the major vector in the city.

In 1977 from January to November, over 1,75,000 parasite positive blood smears from fever cases were detected (Pattanayak, 1977). It was, however, estimated that roughly at least 400,000 cases had occurred in that year (Ramachandra Rao, 1979). Quite obviously the kind and proportions of malariogenic factors in Delhi have changed considerably since the pre-DDT period and a revision of the programme of anti-mosquito measures has become necessary.

Bangalore City

There are records of urban malaria control in several other cities. Even Bangalore city, now considered to be free of locally acquired malaria, was, it appears, quite malarious at one time. Sweet and Rao (1934) have described the situation with a spleen rate of 23.2 per cent for the whole city and parasite rates ranging from 6.5 to 21.2 per cent in different parts of the city. They regarded *A. stephensi* as the main vector breeding in wells, of which there were 3,300 in the city. They instituted anti-mosquito measures including the use of *Gambusia* fish, and in a few years brought the incidence down to spleen rate levels of less than 1 per cent and parasite rates to about 5 per cent.

Pune City

Pune (formerly Poona) has been notorious for its malariousness for a long time. Surveys by Barber and Rice (1938) and extensive surveys in 1944-45 by the Bombay Malaria Organisation conclusively showed that *A. culicifacies* was the vector. Most parts of the city were malarious, particularly those close to Mutha Mula river and the canals from the Khadakvasla dam. *A. stephensi* of the city were found by Sweet and Rao to be of var. *mysorensis*. It was considered as a secondary vector. In 1944-45, the control of malaria by purely anti-larval measures was started with a modest budget. The method used consisted mainly of the use of paris green or copper cyanide as larvicides once or twice a week. Within two years the dispensary figures fell by 75 per cent and the spleen and parasite rates were reduced to a third or 1/5th of what they were before. Anti-larval measures proved themselves to be quite practical and economical. As the idea of bringing about a still greater degree of control by anti-larval work was under consideration, DDT came into the picture. In 1951-52 the entire Pune city was taken up for DDT indoor residual spraying under the direction of Dr. J.K. Adranvala, the Health Officer of the city. This work resulted in a further dramatic improvement. Incidentally, Pune was the first large city in India, and perhaps also the only one, in which the DDT indoor residual spraying programme for the entire city was taken up. The population of Pune at that time was over 400,000. The scheme continued for many years when no more cases were seen in the city. However, along with other areas in the country, Pune has also now seen the resurgence of malaria because anti-larval measures were practically abandoned.

Work in cities such as Calcutta, Madras and other places was carried out, but with limited success. Malaria is now persisting in Madras city mainly because of the breeding of *A. stephensi* in wells.

Control of Irrigation Malaria

Sarda Canal Project

It has been long known that malaria incidence increases in the wake of new irrigation projects. Among the earliest reports of anti-malaria operations in irrigated areas was one by Clyde (1931) during the Sarda Canal construction between 1920 and 1929. The project was mainly aimed to irrigate extensive areas in Uttar Pradesh. The canal headwaters were situated on the Sarda river in Banbasa near India-Nepal border. The project envisaged 4,000 miles of canal system and about 1,800 miles of drainage channels. The area commanded was 7 million acres and the labour required was an average of 10 to 15 thousand men in each of the 13 divisions into which the project was divided. After the preliminary survey of malaria and anophelines, several types of anti-malaria measures were undertaken, viz., jungle clearance, drainage schemes, use of oil, use of Paris green, filling of borrow pits, use of larvivorous fish, and fumigation of temporary and permanent buildings once a week by the use of cresol, carbolic acid, sulphur, pyrethrum and tobacco leaves. Routinely, a mixture of equal parts of sulphur and powdered waste tobacco leaves was used and it was stated that the fumigation was of great value. But in the labour huts where people objected to fumigation, pyrethrum or cresol was used as sprays. The most efficient spraying mixture, however, was carbon tetrachloride 1% and cresol 2% in 97% of kerosene oil.

Not all of the measures were successfully implemented. It cannot be stated that malaria was very effectively controlled; but there was a significant fall in malaria sickness rate between 1924 and 1927. There was a recurrence or an epidemic in 1928 due to "exceptional weather conditions". However, the work was an example of an early and sincere effort to control malaria in an irrigation project. The importance of the work lay in that it was recognized that irrigation and aggregation of labour could lead to a malaria problem.

The Water Logging Board of Punjab in a note published in 1930, defined the principles to be adopted in the preparation of canal projects and their execution (Anonymous, 1930). Sensible recommendations were made for the prevention of mosquito breeding such as lining canals, proper drainage, and proper construction of weirs. Borrow pits, if necessary, were to be dug within the canal beds, main canals and branches were to be at least 2 miles away from any existing town, no new town was to be built within 3 miles of the canals, and carrying out soil surveys and all new canal projects was to be placed before the Water Logging Board for prior scrutiny.

Mandya District, Karnataka

Better success was achieved under the Irwin Canal Project (now Visveswaraya Canals) in Mandya irrigated area of old Mysore State. Very extensive investigations

were carried out by Dr. W.C. Sweet and Dr. B.A. Rao between 1928 and 1935. The vector in that area was *A. culicifacies* with *A. fluviatilis* acting as a secondary vector. Many types of control measures were instituted including prevention of irrigation within 1/4th mile from villages, rotation of crops, use of Paris green in addition to chemotherapeutic measures (B.A. Rao, 1945). During a period of five years, in a group of 10 villages, Paris green was found to be effective for the first four years when there was absence of extensive paddy cultivation within 1/4th mile zone around villages. However, when rotation of crops was practised in the fifth year, making it possible for fresh transplantation of paddy within the 1/4th mile zone, Paris green alone was unable to control malaria. The incidence of malaria was greatly reduced by anti-larval measures. However, the introduction of pyrethrum spraying in the early 1940's and DDT in 1947-48 dramatically improved the situation and anti-larval measures practically ceased to be in operation since then.

The following measures were adopted to prevent malaria making its appearance in village which were to get irrigation water under the project (B.A. Rao, 1948). A committee of officers of health, engineering and revenue departments was formed to investigate the problem and to suggest permanent measures.

The committee started work in April 1941 and drew up recommendations for about 479 villages for permanent improvement chiefly aimed as a permanent solution of the problem of malaria without unduly interfering with the development of irrigation under the project. The engineer on the committee prepared the projects for drainage (irrigation).

The chief recommendations of the committee were:

1. The creation of a dry belt of 2 furlongs around each village.
2. Depletion of all tanks in the area with the exception of a few major tanks.
3. Canalization of valleys which were silted and choked with vegetation, providing them with suitable gradient and sections to carry the seepage and irrigation surplus water.
4. Deviation of channels outside the dry belts wherever possible or lining them with cement concrete within the dry belt in other cases.
5. Shifting of small hamlets and villages adversely situated to the nearest big villages, where the above protective measures have been provided.

These purely preventive measures were adopted in 200 villages, before water was allowed in the irrigation channels, with 'excellent' results. These villages even after having had the benefit of irrigation for over a period of 5 to 6 years, did not experience malaria. No other anti-malaria measures were undertaken in these villages. But for these timely preventive measures (the entire area which was potentially malarious) this area would have developed acute problems following new irrigation. The economic advantages of the measures have been well described by Bhombore *et al.* (1952).

Covell (1947) reports the proceedings of a committee appointed by the Government of India regarding the policy to be adopted for prevention of malaria during railway and road construction.

Cauvery-Mettur Project

Yet another example of control of *A. culicifacies*-transmitted malaria in an irrigated area by anti-larval measures, was by Knipe and Russell (1942) during a period of four years.

A formerly non-malarious area, Pattukkottai Taluk of Thanjavur District, in erstwhile Madras State, became a malarious area in 1934-1935 following the introduction of a large irrigation project called the Cauvery-Mettur Project. Well meaning and extraordinarily efficient engineers brought in water to convert the barren land into a veritably granary. But in its earliest stages, all the fundamental principles of malaria prevention were ignored except at the Mettur dam site. The question posed by the engineers was, "Why should there be malaria due to irrigation in Pattukkottai while hardly 30 miles away the same Cauvery water has been irrigating thousands of acres of rice in the old Tanjore delta for over a thousand years?" The situation in Pattukkottai was shown to be due entirely to "untidy irrigation" (Russell, 1938). Attempts to correct some of the defects and deficiencies were made by the Malaria Investigations of South India of the Rockefeller Foundation in cooperation with the Madras Public Health Department between 1936 and 1942.

Control by anti-larval measures was demonstrated in six villages. The total population of all the villages was approximately 3,400 and the area was about 7 square miles. Within the area were 27 tanks of various sizes and the area was criss-crossed by numerous main, branch and field canals. There were over 1,300 irrigation wells (relics of the pre-irrigation period) and 150 domestic wells. There were numerous rice fields. In addition to many fields lying along branch canals and field channels, there were numerous borrow pits dug during construction of canals and filled by seepage or waste irrigation water. Reduction in malaria incidence was brought about by consistent use of the following methods:

1. Filling up of all borrow pits using earth taken from fields too high to be irrigated thus bringing more land under cultivation.
2. Realigning and straightening field channels.
3. Improving the canal bunds to prevent overflows.
4. Intermittent irrigation of the fields.
5. Use of Paris green both by hand dispensers and automatic machines.
6. Introduction of *Gambusia* fish in wells and tanks.

At that time, i.e., 1937 to 1941, the total cost for 7 square miles was Rs. 14,841/- and the cost per capita was Rs. 4.60. As these measures were carried out in the worst type of rural malaria situation, the cost of Rs. 4.60 per capita was not considered high for a four year period (annual cost Rs. 1.15 per capita). Further, many of the measures led to permanent improvement of the environment. The present day cost (1979) would of course be much higher but if distributed over a period of years in a programme of gradual improvement of the environment, the cost would come within feasible limits.

What was demonstrated was that it was not irrigation *per se* which was the cause

of malaria, but untidy and ill-organised irrigation leading to much wastage of water. It had been predicted that when the entire area was completely under full irrigation, without waste of water, malaria would automatically come down, a prophecy which was soon proved to be true, following the loss of Burma and the subsequent stoppage of rice imports.

Control of Rural Malaria

It is, however, the control of malaria in rural areas which has posed the greatest challenge to the malariologist. While it had been recognised for some time that elimination or control of breeding places upto about half a mile beyond a village would give useful results, the possibility of undertaking work on a grand scale never had been considered seriously either by the public health authorities or the administrators. Such programmes were thought to be impracticable. Only in certain types of special situations, as in the tea plantations, during construction of railways, some major irrigation project sites, where feeble attempts being made. The economic aspects far outweighed the technical ones. Stray attempts had also been made in Bengal by enthusiastic laymen to clear vegetation from ponds, lakes, etc., on a co-operative basis, coupled with experiments to increase fish culture under enthusiastic men like Dr. G.C. Chatterjee. But lack of enthusiasm on the part of the Government and also the people left the rural malaria situation where it was.

Some of the major efforts which yielded good results will now be mentioned.

North Kanara District, Karnataka state—control of rural malaria caused by *A. fluviatilis* by anti-larval methods

The Kerala workers, namely Vedamanikkam and others, have demonstrated that if channels and streams can be kept free of vegetation, *A. fluviatilis* breeding could be eliminated. But large scale efforts had not been made in the difficult terrain where *A. fluviatilis* breeds in the Western Ghats. When the Bombay Malaria Organisation started its work, in 1942, in North Kanara district, vigorous attempts to control the species were made. The failure of pyrethrum spraying is described elsewhere. Simultaneously methods using anti-larval measures were also initiated. The present author and Viswanathan carried out many investigations which have been reported by Viswanathan (1946*a* and 1950).

The measures adopted in 13 towns and villages were:

(1) Clean weeding of all streams and channels for a distance of half a mile of the periphery of villages. The total length of such breeding places was about 6 to 8 miles per village. This work kept the breeding places completely free of *A. fluviatilis*.

(2) The use of Paris green or Copper cyanide mixed in road dust. This was adopted primarily in rice fields and small streams and channels which could not be clean-weeded for various reasons.
(During war-time, Paris green became scarce and Copper cyanide was substituted after ensuring that it was at least as safe as Paris green).

The work was continued for about two years. Moderately good results were obtained. For example, in Haliyal town, with a population of 7,000, the spleen rates were reduced from 62 to 32 per cent, parasite rates from 22 to 14 per cent, and malaria morbidity figures from an average of 2,600 to 1,380 cases (about 48 per cent reduction) within one year. However, the experiments were abandoned in 1946 after DDT came into use.

Malaria Control in Assam Tea Gardens

In Assam tea gardens Ramsay and his co-workers successfully prevented the breeding of mosquitoes, especially *A. minimus*, by growing shade-giving plants over the garden drains, small streams, ditches and swamps. But this method was later given up, because the bushes used for shading began to invade tea gardens, in favour of anti-larval measures and oiling (Covell, 1955). In addition, sluicing and flushing siphons were also put into practice. These measures were very successful and there was significant reduction in malaria indices. During World War II, the larval control methods received set-backs, and by 1944 malaria indices were back to pre-control levels (Gilroy, 1958).

Viswanathan tried the use of pyrethrum space spraying for the control of *A. minimus* on an experimental basis in selected areas. He reported satisfactory results.

Residual spraying with DDT and BHC was undertaken in the tea estates after the War and was supplemented with suppressive treatment (Proguanil). This resulted in remarkable reduction in malaria, surpassing the success of early larval control. After some experimental field trials it was observed that BHC was more effective than DDT on mud walls. Hence use of BHC at a dosage of 11 mg of gamma isomer per square foot at intervals of not exceeding six weeks was recommended for use in the tea estates (Gilroy, *loc. cit.*).

Malaria Control in Bengal-Nagpur Railway

Another example of malaria control in rural areas mainly by the use of anti-larval measures was the work of Senior White and colleagues in the erstwhile Bengal-Nagpur Railway (now S.E. Rly.). The railway passed through some of the most malarious areas in east central India, including parts of Bengal, Bihar, Orissa, Madhya Pradesh and Andhra Pradesh. The efforts were directed mainly to protect the staff of the railway located at different stations. The anti-larval measures consisted of filling up of borrow pits, canalisation and use of Paris green and oil. As in most other rural areas, modest results were achieved. Pyrethrum spraying failed in this area which was dominated by vectors like *A. fluviatilis* with *A. varuna* and *A. annularis* as secondary vectors. *A. sundaicus* was also an important vector in certain sections particularly in Orissa coast. But its control by anti-larval measures was not satisfactory as desired. Venkat Rao (1949b) did an experiment of removing vegetation from tanks and ponds and obtained some success against larvae of *A. sundaicus*. However, the advent of DDT put a stop to all further studies of anti-

larval measures. Though anti-larval measures were being undertaken with some success during the construction of the Vijayanagaram-Raipur Railway, which passes through one of the most malarious areas in the country, it was finally DDT which made the completion of the project possible.

Malaria Control in Coal Fields

Coal fields have received special attention regarding malaria control. The earliest control operation was in Raniganj Coal Field (West Bengal) in the year 1931. It consisted of small scale anti-larval work. Later malaria control measures were soon extended to ten more coal fields in India. The early control methods were mainly anti-larval, consisting of application of Paris green, oiling, deweeding, canalisation and minor engineering works. Later on spray-killing with pyrethrum was done on a limited scale. The work resulted in a moderate degree of reduction in malaria incidence. But when DDT was introduced from 1948 onwards, the history became similar to that of the rest of the country (Sen and Azeez, 1963a).

Spray Killing of Adult Mosquitoes by Pyrethrum

Extracts of pyrethrum flowers *(Chrysanthemum cinerariaefolium)* have been used for centuries to kill insects, particularly in Japan and Iran. There has been a reference to it in the *Arabian Nights*. Because of its knock-down effect, it has been used for a long time in household sprays against domestic insect pests. As far back as 1911-12, pyrethrum extracts had been used in Sudan and later by the British Army during the First World War. Pyrethrum had been used even in the Sarda Canal Project in 1920-29 (see above). However, the use of pyrethrum extracts as a major means of control of malaria had not been explored till the 1930's when Park Ross and De Meillon of the South African Institute of Medical Research presented their experience in killing vector anophelines with pyrethrum-petroleum sprays. Ross had used pyrethrum extracts in 1930-31 against *A. funestus* and *A. gambiae* in Natal and Zululand. The results were so good that by 1933-34 the use of pyrethrum had become a standard measure. In India, workers of the Malaria Institute, under the guidance of Covell carried out in 1934-35 the first field trial on a low scale (Covell, 1941a) with some success. It was, however, the workers of the Rockefeller Foundation (Russell and Knipe, 1939, 1940 and 1941 and Russell *et al.*, 1943) who carried out extensive studies for four years on the use of pyrethrum space sprays which demonstrated its utility for the control of malaria transmitted by *A. culicifacies* in Pattukkottai. Their well documented reports gave a tremendous impetus to the use of pyrethrum sprays all over the world, as a means of control of rural malaria, which till then had been considered as not economically feasible. The spraying was done once a week during day-time in the houses and cattle sheds of villages. Pyrethrum sprays were dispersed as mists by use of hand or power sprayers. The sprays gave excellent results inspite of the fact that the houses were constructed out of loosely woven coconut leaves and could never be tightly closed. This work (with which the present author was intimately associated) cost hardly a quar-

ter of a rupee per capita when water emulsions of pyrethrum extract (Russell, Ramachandra Rao, and Knipe) were used. Covell has elsewhere admitted that he was extremely sceptical initially of the spray killing method, but later became a most convinced advocate.

Pyrethrum sprays were extensively used by the Allied Armies in the different theatres of the Second World War, including the Burma front (Afridi, 1962). In India good results were obtained by pyrethrum extracts in many places, including Mandya district of the old Mysore State by Dr. B.A. Rao. Viswanathan (1941) applied the pyrethrum spraying method in Assam against *A. minimus* and reported promising results.

In the North Kanara District (now in Karnataka) the present author carried out several experiments on the use of pyrethrum against *A. fluviatilis* with disappointing results. This led to a series of studies on the bionomics of *A. fluviatilis* particularly regarding its biting and resting habits and the gonotrophic cycle. Viswanathan and Ramachandra Rao (1943) and Viswanathan, Ramachandra Rao and Rama Rao (1944) have referred to these studies and attributed the failure against *A. fluviatilis* malaria mainly to the outdoor resting habits of the species. They estimated that about 60 per cent of the females rested outdoors during warmer months and about 40 per cent in the colder months of the year. The species was highly anthropophilic and had natural plasmodial infections upto an average of 9 per cent (sometimes even as high as 40 per cent in some localities). Therefore, spraying of pyrethrum once a week in the houses made no impact on the malaria situation. Even twice a week spraying had not been of help. Viswanathan *et al.* (*loc. cit.*) therefore, calculated the best method of applying pyrethrum space-sprays consistent with economy and suggested a 4-days-a-week spraying schedule with proper spacing (two consecutive days of spraying followed by intervals of 2 and 1 sprayless days). Before this method could be tested, DDT became available and further studies were not carried out.

Senior White and colleagues in east central India, particularly in the railway quarters and neighbouring villages, used pyrethrum spraying with equally disappointing results. The major vectors in that area were *A. fluviatilis* and *A. varuna,* and a minor vector was *A. annularis*. Both *A. fluviatilis* and *A. varuna* had outdoor resting habits and were not amenable to control by pyrethrum space-spraying. Senior White (1945) making a thorough review of the subject suggested that in that area perhaps a '6-days' spraying per week might give reasonably good results. Even this was not tested either for effectiveness or for economic feasibility because DDT supplanted all other methods at that time.

De Burca (1939) recorded that pyrethrum spraying of the barracks in certain cantonments in Pakistan was responsible for the reduction of malaria in 1938.

It is worth noting that pyrethrum spraying has its good as well as weak points. Against *A. culicifacies*-transmitted malaria of short duration, it is likely to be quite effective and economically feasible; while against species such as *A. fluviatilis, A. varuna* and *A. minimus* it cannot be efficient. In large parts of the country, particularly in the plains of India, where malaria is transmitted by *A. culicifacies,* the

utility of pyrethrum becomes quite obvious. There are many rural areas in the country in which its use being economically feasible will merit serious consideration and adoption. Much malaria in India can be controlled by re-introducing pyrethrum sprays.

The manner in which pyrethrum extracts were used in malaria control projects may now be briefly described.

Pyrethrin, the toxic component of the pyrethrum flower, is a nerve poison. Because of the rapidity of its absorption almost all species of insects will be knocked down as soon as they come in contact with it. With a suitable dose the insects rarely recover, particularly a frail insect like the *Anopheles* mosquito. It is a contact insecticide and when spraying is done in the form of a fine mist inside houses the droplets become dispersed and penetrate into all nooks and corners of the room and even through the coconut leaf matting, the mosquitoes which come into contact with the droplets die immediately. There is an optimum size of droplets. If they are too fine as in a fog, they are likely to go around the insects and not impinge on them, enabling them to escape death. For this purpose some of the usual hand pumps like the flit-gun or the mist sprayer developed by Puri and widely used by the Indian Army or even the two types of hand sprayers developed by Knipe (like the "Arrow" and "Cobra") are very efficient. Russell and Knipe used very effectively paint-spray guns giving droplets of the proper size. Pyrethrins have no residual effect and are quickly decomposed on exposure to sunlight. It is, therefore, obvious that pyrethrum space sprays can kill only those mosquitoes which are actually resting inside houses or cattlesheds at the time of spraying. The effect can be improved by closing all doors and windows for a short period after spraying. The large number of mosquitoes resting outdoors, or those which enter dwellings later, are not affected. With spraying done once a week only a small proportion of adults are killed. Still very good malaria control was obtained against *A. culicifacies*. This can be attributed to the following factors: (*a*) the proportion of *A. culicifacies* resting indoors during daytime is high; (*b*) *A. culicifacies* is predominantly a cattle-feeder and therefore has a low infection rate and the numbers positive at any given moment are rather small; and (*c*) though a few infected specimens may escape being killed on the first occasion, the chances of their biting man again are very meagre. Added to it is the fact that *A. culicifacies* is comparatively, even normally, a short lived species and the proportion which becomes sporozoite positive is very small. An advantage of pyrethrum is that till now there is no evidence of the development of insecticide resistance to it in mosquitoes. Moreover, it is absolutely safe to humans and animals.

DDT ERA

The next major development in the control of malaria vectors in India was the introduction of DDT as residual insecticide in the yeaf 1944. While space-spraying with pyrethrum kills the mosquitoes present in dwellings at the time of spraying, residual insecticides work by remaining as thin deposits on the wall, roof and other

surfaces inside dwellings for several weeks and killing the mosquitoes which come to rest on such surfaces, by contact through their tarsi. These insecticides are so potent that even a slight contact with the tarsi is enough to kill the insects. Adults of some species of anophelines may exhibit the phenomenon of excito-repellency and because of the irritation caused by the insecticide may leave the sprayed structures even before feeding on the host. It is enough if the residual insecticides are sprayed once in several weeks, or even months, and the deposits remain for such periods. This brought down the cost of control to very low levels. All that was necessary was for the females of the vector species to come to rest on the sprayed surfaces before, after or both before and after taking their blood meals. The control of malaria was obtained by the reduction of the length of life of the vector species. If they were not killed on the first occasion of entry into premises they had further chances of being killed on the second and subsequent feedings and consequently, only a few females lived long enough to reach the dangerous age of 9-11 days.

The use of residual insecticides revolutionized malaria control all over the world. The dramatic results which followed led to the hope of complete eradication of the disease for which large scale programmes were set in motion.

DDT (Dichloro-Diphenyl-Trichloroethane) had been synthesized as early as 1874 by an Austrian chemist, Othmar Zeidler; but it was Paul Muller, a Swiss chemist, who resynthesized it and discovered its remarkable insecticidal value in 1939 in Basel, Switzerland, for which he received the Nobel Prize in 1948. Its first use was for moth-proofing of woollen cloth. Once impregnated, the cloth was completely resistant to the infestation by the moth. Later it was used in agriculture as a dust known as 'Gesarol' and also as a dust mixed with inert substances like talc (known as 'Neocid) for killing body lice. It was in 1942 that its utility as an anti-mosquito agent was discovered by the U.S. army. Extensive researches were done on the substance in U.S.A. and U.K. and the anti-mosquito effect was demonstrated effectively by the Allied Armies in 1944 at Volturno in Italy (Russell, 1955). Also for the first time in history and epidemic of typhus in Italy was halted during winter by the use of DDT.

DDT was introduced in India in 1944 by the British and American armies on an experimental scale on the Burma front (Afridi, 1962) and small quantities were released for civilian use by General Covell. Among the first to use DDT in civilian practice in India, almost simultaneously in 1945, were Senior White (1945) and Viswanathan and Parikh (1946). A few laboratory and field tests had also been made on insects of medical importance at the Calcutta School of Tropical Medicine (Wu *et al.*, 1946). Senior White tested DDT in comparison with pyrethrum sprays in a few villages in Orissa, but Viswanathan and Parikh tried it on a larger scale in 41 villages of Sirsi Taluka in North Kanara. Viswanathan's work was made possible because of the initiative and foresight of General Covell in diverting about two tons of technical DDT meant for the army for the use of the Bombay State (Covell, 1955). The density of *A. fluviatilis* adults was reduced from 2.8 for 10 man hours to 0.08 for 10 man hours in the sprayed houses.

The results of the preliminary work were so spectacular that the Bombay Govern-

ment, urged by Viswanathan and fully supported by General Covell, lost no time in organising the largest rural malaria control programme organized till then, commencing in July 1946, in two districts, North Kanara and Dharwar, seeking to protect a population of over one million living in highly malarious areas. The inauguration of the project received a telegram of blessings from Mahatma Gandhi. The plan of operations and results of the programme have been described in detail by Viswanathan and Ramachandra Rao (1947, 1948 and 1949). DDT was used as an emulsion at a dose of 56 mgm/sq. foot at intervals of 6 to 8 weeks, thrice during the malaria season. The results were unbelievably good. The spleen rates, parasite rates, infant parasite rates and dispensary figures showed spectacular reductions.

Experiments with DDT were also commenced in Quetta (Afridi and Bhatia, 1947), in Delhi province (Afridi and Dilip Singh, 1947), in Delhi (Puri, 1948), a pilot demonstration in Puttur Taluk, Karnataka (Ramakrishnan *et al.*, 1948), in Mysore State (B.A. Rao), in many places of the then Bengal-Nagpur Railway (Senior White and colleagues), in Bengal (Kar, 1950), and by many other Central and State organisations, all reporting uniformly good results. Excellent reports were also received from many cantonments.

By 1953 DDT had become the sole method of malaria control in many places in India and large areas had already been brought under very effective control. In fact, by the year 1952-53, the entire malaria endemic areas of the old Bombay State (a population of nine million out of a total of 18 million) had been protected by DDT spraying schemes. The spleen rates, parasite rates, infant parasite rates and dispensary statistics were reduced with uniform success everywhere. (For a general and popular account of the genesis of the N.M.C.P., read Viswanathan, *Conquest of Malaria*, 1957).

Extensive programmes had also been launched in most malarious countries of the world except in tropical Africa, and malaria was nearly eradicated in several European countries. Venezuela was probably the first to launch a countrywide programme in 1946 under the leadership of Dr. A. Gabaldon.

Between the years 1949-53 the World Health Organisation established several malaria control demonstration teams for the use of DDT. In India four such units were started, in Sagar area of Karnataka, in U.P. Terai, in Orissa and in Wynaad Taluk of Kerala State.

National Malaria Control Programme, 1953-1958

Based on the experience of eight years in different parts of India, the Government of India, in co-operation with all the State Governments and the U.S. Technical Co-operation Mission, embarked in 1953 on a National Malaria Control Programme (NMCP) for protecting the entire malarious area of the country. The objective was to reduce the incidence of malaria to very low levels. This 5-year programme achieved ramarkable success (B.A. Rao, 1955; Jaswant Singh *et al.* 1957), and it appeared that the next logical step should be an attempt completely to

eradicate the disease from the country. Rumblings of the development of insecticidal resistance in certain vectors in some parts of the world, including *A. stephensi* in India, were audible and urged the malariologists and administrators to attempt a total eradication of malaria before the resistance phenomenon became a real threat to the programme.

National Malaria Eradication Programme (NMEP)

Again the Government of India, with the collaboration of the USAID, WHO, UNICEF and all the States in the country launched in 1958 a National Malaria Eradication Programme (NMEP) originally with a five year plan (extendable if necessary). The organisation and the achievements of these two programmes have been very well documented and need not be repeated here except briefly.

The N.M.E.P. differed from N.M.C.P. in having to include even low endemic and potentially malarious areas in the programme and setting up a surveillance organisation during the consolidation phase to cover the entire population of the country. Cities and towns with a population of over 40,000 were excluded from the programme because anti-larval measures were regarded as economically feasible in them. Some spraying of peripheral areas was however allowed.

There were ultimately 392.35 NMEP units in the country and a phased programme of withdrawal of spraying was envisaged. However, areas equal to 25 units in the border regions were to be sprayed indefinitely to provide a cordon sanitaire. It was later expected that malaria would be eradicated from the country within a phased time schedule of seven to nine years.

Essential features of an eradication project

Any national programme for the eradication of malaria as recommended by the W.H.O. was to have four phases:

(*a*) preparatory, (*b*) attack, (*c*) consolidation, and (*d*) maintenance.

In the *preparatory phase* thorough malaria surveys, including detailed surveys on vectors and their bionomics, had to be undertaken and arrangements made for the spraying programmes. In the *attack phase* residual spraying in all man-made shelters with insecticides like DDT, HCH, etc. was to be done. The attack phase was to last three years during the last of which, in addition to spraying, a surveillance machinery (active as well as passive) to detect residual cases of malaria and their radical treatment was to be set up. The *consolidation phase* was to commence after three years of attack and spraying operations were to be suspended and surveillance was to continue when certain set criteria were achieved (0.1 cases per 1,000 people); that is, when the annual parasite incidence reached the required level. The area was next to go into *maintenance phase* on the basis of further criteria. During this phase spraying was to be discontinued except in selected localities and the vigilance was to be continued by the general health services of the district which was to take over all the responsibilities.

The basic idea was that malaria transmission should first be greatly reduced and the remaining few cases of malaria be sought after and radically cured.

In the Indian programme the preparatory phase was largely omitted since it was believed that ample data and information on the malaria vectors had already been gathered for nearly eight years including five years under the N.M.C.P. The attack phase was launched in the year 1958. During this phase spraying of all structures throughout the country with DDT was planned. DDT 75 per cent wettable powder was sprayed at the rate of $1g/m^2$. Generally, two rounds of spray per year were given, though in certain areas, where the transmission season was prolonged, three rounds were applied. It was impossible to obtain 100 per cent coverage of the human dwellings for various well known reasons; often the coverage was only 50 per cent of structures. Yet the incidence of malaria came down in most units. During the consolidation phase, was set up a surveillance machinery which consisted of surveillance workers and inspectors who visited each house in every village of the country once a fortnight to detect fever cases. In addition passive surveillance by all government hospitals and dispensaries and private agencies was also organized. All fever cases detected were given presumptive treatment with 600 mgs chloroquine (adult dose, suitably reduced for children) after taking a blood smear. The slides were to be examined as early as possible and every parasite positive malaria case was to be given a radical treatment with a single dose of chloroquine, and primaquine for five days at 15 mgs per day. The examination of blood smears was in many cases unduly prolonged leading to backlogs.

For the entry into maintenance phase the Indian programme generally followed the criteria set by the W.H.O. : (1) proof of adequacy of surveillance machinery under the general health services, (2) evidence that in the period of three years no indigenous cases had been discovered, (3) evidence was established beyond reasonable doubt that any malaria case detected was either imported or a relapse of a pre-existing infection and not directly secondary to an imported case; (4) laboratory services should be adequate. Certain administrative aspects had also to be considered, including the adequacy and ability of the general health services to take care of any positive cases which may occur. Unfortunately, for various administrative reasons, these criteria could not be strictly adhered to.

Annual appraisals of the programme were done for the units, which were candidates for conversion, by independent teams of experts which made the necessary recommendations. It has to be admitted that conversion to the maintenance phase was made prematurely in some units.

From 1964 onwards, due to the occurrence of small outbreaks in several units, the smooth transfer of units from attack to consolidation and consolidation to maintenance was upset and many units which were already in maintenance had to be reverted to the attack phase. Certain re-phasing of the units became necessary (Dhir, 1968). By 1974, it was realised that the approved phasing pattern was no longer valid, and a modified plan was initiated from 1977. According to this modified plan, insecticidal spraying of DDT, HCH or malathion was to be made only in selected localities where the insecticides were effective and main reliance was to be

placed on intense drug distribution, to keep morbidity down and to prevent mortal ity completely.

The NMEP of India which has been rightly described as the single largest public health programme in the world, received much technical support from the W.H.O. and international malariologists. Liberal aid in the form of equipment and insecticides were made available by USAID. Because of the vastness of the programme, the number of agencies involved and the difficulties of logistics the programme took a couple of years to become fully established. Even so, spectacular reduction in malaria incidence occurred in practically all parts of the country. The number of registered malaria cases proved by parasite detection came down from an estimated 75 million prior to DDT programme to less than 2,00,000 in the year 1965. But from that year onwards the number of malaria cases detected gradually increased year by year. In 1977, the registered number was about 6 million. This set-back has now been recognized as due to administrative weaknesses as well as some important technical features. It should be appreciated that the resurgence of malaria has occurred not only in India but in many parts of the tropical world including all neighbouring countries. The numbers of malaria cases detected during surveillance in India (from NMEP sources) were as follows:

Year	Malaria cases	
1961	49,151	Surveillance not fully established.
1962	59,575	
1963	87,306	
1964	112,942	
1965	100,185	
1966	148,156	
1967	278,621	
1968	274,881	
1969	348,647	
1970	694,647	
1971	1,323,118	
1972	1,362,806	
1973	1,930,273	
1974	3,167,658	
1975	5,166,142	
1976	6,166,847	
1977	4,740,900	
1978	4,144,385	
1979	3,064,697	
1980	2,896,000	
1981	2,666,244	
1982	2,160,447	

Several reviews of the NMEP have been carried out. An early one in 1960 was by

a team led by Dr. Harold Hinman. Later after setbacks had occurred several technical reviews have been made, the chief among them were:

(1) A special committee to review the working of the NMEP and to recommend measures for improvement, headed by Mr. R.N. Madhok, in 1967.
(2) The Indepth Evaluation Team of nine international experts from WHO and USAID, with Dr. T. Ramachandra Rao of India, as the Leader in 1970 (appointed by Government of India).
(3) The second indepth study made by a small committee headed by Dr. M.I.D. Sharma in 1974.
(4) Again a committee of experts, who in view of the great urgency of the problem and shortage of funds, reviewed the entire programme as an emergency in 1974 under the chairmanship of late Dr. B.A. Rao. This committee suggested a modified programme for the N.M.E.P. which has been implemented since 1977 by the Government of India with a few important modifications (Pattanayak, 1979; Pattanayak and Roy 1980). While complete malaria eradication was set as the ultimate goal, the immediate objective was to be a good control programme feasible in the current circumstances. The first objective was to prevent a further escalation of the disease and to reduce morbidity and prevent mortality.

The reports of these teams are unpublished documents in the records of the Government. The W.H.O. Expert Committee of Malaria in 1974 (WHO, 1975) also has stressed the need to change the immediate objectives of national programmes to one of good control of the disease in areas where eradication was not feasible for any reasons.

The reasons for the setback in the N.M.E.P. in India and in other countries have been pointed out by several authors particularly by Ramachandra Rao (1979), Ray (1977) and Kalra (1978). Several reasons have been adduced. The major ones as seen by the present author are:

(*a*) Widespread resistance of the major species of malaria vectors, such as *A. culicifacies* and *A. stephensi*, to insecticides DDT and HCH and in some places even to malathion;
(*b*) Complacence in administrative matters when the incidence of the disease was at the lowest in 1964, 1965 and 1966 and slackness in appreciating the gravity of the situation and in allocation of funds and procurement of materials;
(*c*) Persistence of malaria transmission in certain areas such as in parts of Orissa, Gujarat, Madhya Pradesh, Maharashtra and eastern India due to;
(*i*) incomplete or untimely spraying of insecticides.
(*ii*) refusals from householders for getting their premises sprayed.
(*iii*) the role of vectors such as *A. balabacensis* which had not been previously recognised due to their exophilic and exophagic habits.
(*iv*) chemotherapeutic measures could not give that degree of complete radical cure as was expected and there were breakthroughs of a number of

infections enough to create fresh foci;

(*d*) Very rigid criteria were not applied for the conversion of the N.M.E.P. units to go into the maintenance phase resulting in less than adequate attention being given to vigilance against malaria by the District Health Organisations in turn resulting in outbreaks in several places.

There may be some slight differences of opinion as to the relative importance of these causes. At any rate the result has been a resurgence of malaria, which is no longer easily controlled by insecticides. The main reliance in the modified plan has been placed on chemotherapy, both as a preventive and curative measure.

The most important *entomological* factors involved in the setback have been:

1. Insecticide resistance to DDT, HCH or to both and sometimes even to malathion in *A. culicifacies* and *A. stephensi.*
2. The now recognized role of *A. balabacensis* which is an outdoor rester and largely an outdoor biter.
3. The recognition that some species like *A. philippinensis* which were formerly regarded as indoor resters and indoor biters, behaving differently.

The aspects of mosquito behaviour in relation to malaria transmission have been dealt in details in the appropriate sections dealing with individual species.

Residual Insecticides Used in India

DDT (p.p—dichloro diphenyl trichloroethane)

When malaria control with DDT was first started in India, 5 per cent solutions of technical DDT in kerosene were used as in the experiments of Viswanathan and Parikh and Senior White. Later when large scale programmes were started, the formulation used was a water emulsion of DDT prepared from 40 per cent solution of DDT technical in medium kerosene extract (later given the trade name 'Aromex') and emulsified with ordinary washing soap. The spraying was done at a dosage of 56 mgs technical DDT per sq. foot at intervals of 6 to 8 weeks during the transmission seasons. In some localities a dosage of 112 mgs per sq. ft. was used. For several years in the whole of India, this was the formula adopted. In early 1950s DDT water wettable powder containing 50 per cent or 75 per cent technical DDT became availabe. Because of its great convenience both in transportation and storage it became the formulation of choice. All that was needed was to mix it with the appropriate quantity of water in the field. With the N.M.C.P. and later under the N.M.E.P. a dosage of one gramme per sq. m. was employed for each round. In some inaccessible situations, a dosage of 2 gms. per sq. m. was also used in one round.

BHC

The other insecticide used extensively in India has been BHC. BHC (Benzene Hexachloride) or HCH (Hexachloro cyclohexane) was first discovered in U.K. and was used with the trade name 'Gammexane'. Preliminary experiments had been done in India by Bertram (1950) working with *A. minimus* in Assam. He, and later Hay

Arthur and Gilroy (referred to by Viswanathan *et al.*, 1950) reported that it was quite effective. However, a large scale field experiment was done in Thane District of the old Bombay State (now in Maharashtra) by Viswanathan, Ramachandra Rao and Juneja (1949 and 1950). They made a study of the comparative efficacy of DDT and HCH. Used as a wettable powder, the dosage of 11 mg, gamma isomer per sq. ft. compared favourably with DDT as an emulsion 56 mgs per sq. ft. However, DDT was slightly superior to HCH in some respects. But HCH was found to be a very good substitute to DDT. It is the gamma isomer, which forms about 13.5 per cent of the technical material, that is the active principle. It has now been purified and known as the lindane quality used mainly as an emulsion.

HCH is now being very extensively used under the N.M.E.P. in many parts of the country wherever resistance to DDT has been observed. In several parts of the country the vectors have become resistant to HCH also.

Dieldrin

Dieldrin is an insecticide belonging to the *cyclodine* group to which HCH also belongs. It had been used in the early years in connection with filaria control programmes against *Culex fatigans*. In malaria control also a small quantity had been used, but was soon withdrawn not only because of its toxic effects on man, but also because of the very rapid development of resistance in the vector (Patel *et al.*, 1958). Even only two rounds of spraying in Thane District resulted in a serious epidemic of malaria due to development of resistance.

Malathion; (S-(1,2—di (ethoxy-carboxyl) ethyl) 0,0 dimethyl phosphorodithеonate)

An organophosphorus compound, this insecticide was used as a 25% water wettable powder (Bhatia *et al.*, 1968) and later as an emulsion in Maharashtra against DDT and HCH resistant *A. culicifacies* in 1970. The dosage used was 2 gms per sq. m. The result obtained were satisfactory and malathion spraying has now been extended to several areas in the country (Gujarat, Maharashtra, Karnataka and some parts of Uttar Pradesh) where the vector is resistant to both DDT and HCH.

Fenitrothion; (0, 0-dimethyl 0-(3-methyl-4 nitrophenyl)-phosphorotheoate)

Bhatnagar *et al*, (1974) carried out field trials with fenitrothion against DDT resistant *A. culicifacies* in Sikar area of Alwar District of Rajasthan and found it to be quite effective. On sorptive surfaces the effectiveness lasted five weeks when sprayed at 0.5 g/m^2. Increasing the dosage to 1 gram brought about an extension of the effectiveness by only three more weeks. The relative cost, however, was very high; for example fenitrothion cost 10.6 times more than DDT but was slightly cheaper than malathion which cost 12.7 times more than DDT.

In addition, other insecticides like chlordane, diazinon, DDVP, beytex, abate,

etc. have been used purely in very limited experimental trials but none of them has been adopted yet as a major insecticide in the national programme. Abate however is being used in Salem Town in Tamil Nadu for control of *A. stephensi* larvae in wells.

Naturalistic or Biological Control

Naturalistic control methods involve the utilisation of natural factors in the environment, which are adverse to mosquitoes. Man uses them to suppress or diminish the mosquito breeding, either by enhancing the factors already present or by introducing them into habitats when they are not present. In short it is the method of using nature itself to the disadvantage of the mosquito species without using extraneous physical or chemical methods. There are a wide variety of naturalistic methods, such as shading, clean weeding, flushing, alterations in water levels, enriching or impoverishing vegetation and plankton, increasing or decreasing salinity, changing the suitability of water by herbage packing, increasing the number of natural parasites and predators or introducing them etc. The term 'biological control' has also been frequently used especially when the methods involve the use of parasites and predators. Genetic control is also a method of biological control.

Naturalistic methods are generally economical and have the great advantage of not contaminating the environment with long lasting harmful chemicals. However, they are usually effective only to a moderate degree. They cannot give such perfect control of a mosquito species as chemical methods would do. Moreover, the naturalistic control methods would have to be tailored to suit not only the particular species of mosquito, but also to the type and extent of the breeding places. Naturally these methods require a deep insight into the ecology of the vector.

The use of biological or naturalistic methods of control has vast potentialities. But except in the case of use of fish and some instances of manipulation of the environment such as the shading and clean weeding they have not been applied on any appreciable scale in India. While the principles have long been well understood, their application has not been extensive or effective except in certain limited situations. Given serious thought and consistency of enthusiasm some of these methods can be very successful. Unfortunately because of the availability of other effective methods more generally applicable and less exacting, naturalistic or biological methods have been adopted on a scale worthy of them.

Fish

Fish have been used for a long time to control mosquito larvae. As far back as 1904 fish had been used in Bombay City for the control of *A. stephensi* in wells by Bentley and others (Covell, 1928). During the construction of Sarda Canal Project between 1920 and 1928, Clyde (1931) has referred to the use of larvivorous fish. Prashad Hora and Mukherjee (1937), had prepared a list of indigenous fish which could be considered for use for the control of mosquito larvae. Roy (1938a) had found *Aplochilus panchax* a very useful surface minnow in Bengal, confirming the

findings of Sen (1938).

John (1940) tested *Aplochilus* spp, both in the laboratory and in the field and recommended its use in the old Travancore State. Job (1941) carried out field experiments in borrow pits in Howrah District, Bengal with *Aplochilus panchax* and found that the fish destroyed all larvae and was superior to and less costly than Paris green.

However, it was after 1929 that the use of *Gambusia* became a common practice in India. *Gambusia affinis* had been used since the beginning of the century in the U.S.A. and since 1905 in the Hawaii Islands, from where it was imported into many Southeast Asian countries. The species was brought to India by Dr. B.A. Rao from Italy in 1929. Hardly a hundred fish survived the journey, but when introduced into wells and nurseries in Bangalore City, they multiplied very rapidly. *Gambusia* was later distributed to many parts of India. In the old Mysore State it was very extensively used. It has now become almost an indigenous fish.

Gambusia has proved itself to be a great utility particularly in wells and in cirumscribed breeding place. But they have not proved themselves to be useful in large bodies of water. In Puttukkottai, when the present author and Mr. K.S. Krishnan, under the guidance of Dr. Russell introduced *Gambusia* into many rice fields with the hope of controlling breeding of anophelines in that habitat, the results were unsatisfactory. The fish could not acclimatize itself to rice fields. However, there are many reports dealing with the successful use of *Gambusia* in wells and ornamental tanks in many parts of the country, such as Delhi, Bangalore,Pune, many cantonments, Hyderabad City, Visakhapatnam, Guntur, Salem, Madras City, etc. and it is not possible to refer to all of them.

An excellent recent demonstration of the utility to *Gambusia* was by Sitaraman *et al.* (1975) etc., in Hyderabad City. Sitaraman *et al.* (1976) have also reported good results obtained by *Poecilia reticulatus* (Guppy) against *A. stephensi* in wells.

Recently Rajagopalan and others (Vector Control Research Centre, Pondicherry) have re-examined the use of *Gambusia* and some other indigenous fish and confirmed its utility in wells provided the wells were frequently examined and the fish restocked. Results of intense studies by Reuben and colleagues in Salem City where *A. stephensi* is the vector have been very similar. For some undetermined reasons, *Gambusia* fish did not thrive in the wells of that city. It is also known that *Gambusia* fish are deliberately taken out by people for other purpose and in many instances larger fish devour *Gambusia*. Among the indigenous fish experimented by the V.C.R.C., one of some promise is *Aplocheilus blochii*. However, it is difficult to be mass cultured.

Mermithids

Among other parasites and predators which have been tested is a mermithid parasite, *Romanomermis sp.* It has been mass reared in the laboratory and found effective experimentally against *Anopheles* mosquitoes breeding in fresh waters, but its practical use in the field has been rather disappointing. It would be interesting to

note that certain species of mermithids are known commonly to parasitise *Anopheles* larvae in India and the infected specimens invariably die.

Insect predators

Similarly disappointing has been the attempts to use a notonectid bug, *Anisops bouveri,* which is a natural predator of larvae. It was mass reared and worked reasonably well in the laboratory, but failed to show any promise under field conditions (VCRC Reports).

Other parasites and pathogens

Among many other parasites and pathogens which have been or are being tested in India are:

1. fungal pathogens, such as *Coelomomyces;*
2. bacterial agents, such as *Bacillus sphaericus; B. thuringiensis* local strain, and
3. protozoan parasites such as *Nosema algerae, Thelohania* sp. etc.

All these organisms have been isolated indigenously.

Laboratory investigations on pathogens are in progress at a few centres, particularly at the V.C.R.C., Pondicherry. An interesting finding is that *A. stephensi* infected with *Nosema* tend to be less susceptible to *Plasmodium vivax* infections than those which are not infected (Gajanana *et al.*, 1979).

While the idea of use of parasites and predators may appear very attractive,the technology connected with their mass rearing in the laboratory and their use in the field are far from satisfactory. Much research has still to be done before a safe and practically useful agent or agents can be developed. The agents should not become sources of danger to man, animals, and other non-target species of insects.

Recognising the hazards involved in importing exotic agents, efforts have to be made with indigenously recovered agents only. It is, however, known that biological agents, unless they are extraordinarily efficient and specific to the vector, may have value and usefulness in limited and circumscribed situations only.

Biological agents have a tendency sooner or later to establish a balance with the target organisms and while they continue to be of some value they do not give complete control. If the objective is met the complete elimination of the target insect but a reasonably good control leading to reduction in numbers, biological control methods become practical. However, there have been some excellent examples of good control of the target plant, animal or insect by biological control methods.

Sepcific parasites and predators of exotic origin have a much better efficiency than purely indigenous ones. However, introduction of exotic agents is fraught with danger and such agents should not be introduced without a thorough study of all possible hazards.

Caution is, in fact, necessary even with regard to indigenously found parasites and predators. The WHO Expert Committee on Insecticides (1975) has laid down

certain procedures to be adopted for testing stage by stage before any agent can be taken up for general use.

Shading, etc.

Shading has been used as biological method of control of species such as *A. minimus* and *A. maculatus*. Shading small streams and tea garden drains has been extensively used in the tea gardens of Assam. Plants such as lantana, hibiscus, duranta and tarapet were grown along the streams and channels. Good results have been obtained by this method in minimising breeding of both *A. minimus* and *A. maculatus*. Later the method had to be abandoned because the plants themselves became a nuisance to tea gardens (Covell, 1955).

Bhaskar Rao and Ramoo (1942) have used shading of irrigation channels by certain plants and have obtained good results against *A. culicifacies* in the Pattukkottai area. In their case, perhaps it was not shade *per se* but the mechanical obstruction for egg laying which controlled the breedings. In this connection, the studies by Russell and Ramachandra Rao (1942a) on the effect of mechanical obstruction and shade may be referred to.

Clean weeding

Clean weeding of streams and channels have been successfully used against species such as *A. fluviatilis* (Viswanathan, 1946b) but as channels and streams are not the only places where the species breeds, the procedure by itself has not prevented malaria transmission in North Kanara District. Deweeding has also been successfully used against *A. annularis* and *A. pallidus* (Venkat Rao and Ramakrishna, 1947).

Agricultural practices

The use of intermittent irrigation to control *A. culicifacies* in rice fields was found to be practicable (Russell and H.R. Rao, 1940). A schedule of 2 dry and 5 wet days in a week led to a marked fall in breeding and the method was put to use in the experimental control of malaria in several villages (Russell and Knipe, 1942).

Genetic control

Genetic control of mosquitoes also is a kind of biological control. But except for a few attempts made in experimenting with it in relation to *Culex fatigans* early in 1961 by the workers of the National Institute of Communicable Diseases, Delhi (Krishnamurthy *et al.*, 1962; Ramakrishnan *et al.*, 1962) and extensive studies by the I.C.M.R./W.H.O. Research Unit on Genetic Control of Mosquitoes, Delhi, in 1970-75, there has been no work on any anopheline in the country. A large number of reports of an original nature has emerged from the latter study. A general review has been given by Ramachandra Rao (1974). While genetic control holds out great

promise on the basis of theoretical considerations, the technology involved for application in the field is quite complex and involves a very deep and intimate knowledge, of the ecology and bionomics of the vector. Such knowledge is not generally available today. (see also Chapter 5).

Other methods

Anopheles breeding can sometimes be eliminated from certain types of habitats by packing them with cut plants and shrubs (herbage packing) and trampling upon them (Nagendra, 1947). The present author has successfully controlled breeding of *A. culicifacies* in shallow wells by putting cut pieces of cactus (*Opuntia sp.*) into them. However, such practices often lead to rotting of the cut vegetation and provide ideal habitats for the breeding of noxious mosquitoes like *Culex fatigans*.

Many other biological control methods have been suggested and tested in other countries, such as salinification of water to control *A. sacharovi* (in Albania), prevention of growths of blue green algae in fish ponds to control *A. sundaicus* (in Java,(rapid changes in the water levels of lakes and reservoirs as attempted against *A. quadrimaculatus* (in the southern United States) under the Tennessee Valley Authority etc. may be mentioned. *A. culicifacies* larvae in certain types of streams and rivers was reported to have been controlled effectively by using various types of flushing devices (Worth and Subramaniam, 1940) in Sri Lanka. They were, however, liable to be silted and were not effective in dry weather with very slow flows. Similar studies were made by Ramsay and Anderson (1940) against *A. minimus* in Bengal. An automatic siphon was also developed (Macdonald, 1939).

The booklet on *Anti-Mosquito Measures with Special Reference to India* by Covell revised by Ramakrishnan (1962), the article by Williamson (1949) and the booklet on *Mosquito Control—Some Perspectives for Developing Countries* by the National Academy of Sciences,U.S.A. (1973), describe many interesting and practical methods of naturalistic control.

Future

Serious consideration has now to be given to the methods to be adopted in future for the control of anophelines. The experience we have gained over the last four decades should help us to formulate realistic and practical policies. The most effective method so far evolved has been the use of residual insecticides which gave dramatic results but are now failing us. While one can always hope that research will throw up newer insecticides which are more effective, less prone to induce resistance, less toxic to man and animals and inexpensive enough to be used on a large scale in rural areas, the prospects do not at present appear bright. Research in this direction is continuing. Many new types of substances which either act as direct insecticides or interfere with the physiology and behaviour of insects are being considered, as for example, pheromones, juvenile harmones, growth inhibitors, pyrethroids, bio-degradable organochlorine insecticides, chemosterilants, sex distortors, etc. The use of parasites and predators, other naturalistic methods and even genetic

control are being explored. But it is beyond the scope of this book to critically evaluate their prospects.

However, there is growing recognition of the urgent need to revive some of the practical and economical methods of mosquito control of former years, whose further development and testing were abruptly halted about 30 years ago. Some of these are source reduction, source elimination, use of larvicides, use of pyrethrum extracts,etc. The revival of these methods has been well stressed by the WHO Expert Committee on Malaria (1974). It is also recognized that anti-anopheline measures have to be adopted to suit not only the behaviour of the particular species but also the environment in which they thrive. For this purpose, a thorough understanding of the bionomics and ecology of the vectors has become absolutely essential.

Though insecticides are very valuable and perhaps will remain with us in one form or another for a long time to come, they have certain limitations. With the tendency of insects to develop resistance, there is little prospect of any one insecticide being continuously useful for long periods. Many of them have the tendency to contaminate the environment and to affect non-target insects or organisms. Their costs are rising making them prohibitively expensive.

The whole strategy of control of mosquito-borne diseases, including malaria, in the future, therefore, needs careful and deep thought. Insecticides and drugs are palliative measures, requiring ever recurrent action and subject to the inexorable phenomena of development, tolerance and resistance. Sooner or later public health workers and sociologists would have to seriously consider the improvement of the total environment, both in towns and villages as a means of prevention of disease-producing factors. History and tradition have been greatly responsible for the environment of our towns and villages. The development and growth of towns and villages without proper sanitation, economic and social necessities such as the need to grow wet crops, the need for tanks, wells and reservoirs, bringing in of water for irrigation or domestic use but without proper provision of drainage, and even apathy on the part of the people and the civic administrations have all contributed to the creation and persistance of factors conducive to the prevalence and spread of malaria, filariasis and certain virus diseases.

Water is essential for the life and survival of man and his domestic animals, but can water not be used without creating disease hazards? In most parts of the world inhabited by man it can be done. Elimination of all but the essential collections of water will itself lead to a great reduction in disease. A gradual and sustained programme spread over a number of years with the positive participation of the people should help in achieving such a goal. The economic inputs, if spread over a number of years, should be within the competence of the local authorities supplemented by aids by the State and Central Governments.

There is no argument about the desirability of and the need for malaria eradication as the ultimate goal. However, as has been emphasised by many shrewd observers and based on the experience of the last three decades in many parts of the world, eradication is not an automatic process. Each year's work has to be consoli-

dated in subsequent years by more vigorous work. Slackness or the appearance of a technical problem is likely to lead to reversals. One year's reversal may take the programme back perhaps two or three years. It is not like building a bridge. The foundation can be laid and left for a few years. When funds become available the pillars can be constructed. If still funds are not available the construction of the deck can be taken up years later. Each year's work is a cumulative one and the bridge can be built in phases. In malaria eradication using insecticides and drugs the processes are not similar. Slackness in one year will lead to reverse processes which take us back by two or three years. Further, the success of eradication depends upon numerous factors, administrative as well as technical, which would have to reach the highest degree of perfection, perhaps not attained in any other social programme. Every thought should be given to develop strategies for a gradual and positive reduction of diseases and to take up measures which prevent rather than control diseases.

The motto for the future of *Anopheles* control would seem to be:

1. Do not create mosquito breeding places.
2. Eliminate those which exist, except the absolutely essential water sources.
3. Control the larvae and adults which still occur by all possible means, including insecticides.

This chapter on the landmarks in the control of *Anopheles* could not be closed more appropriately than by a prophetic quotation from Senior White written in 1948 hardly two years after DDT was used and ten years before the National Malaria Eradication Programme was launched in this country:

> "The existence of races of noxious insects immune to various insecticides and the fact that such resistance extends to poisons never normally employed in insect control, foreshadows the coming of a day when the control of malaria by insecticides (including DDT and Gammexane) will fail the hygienist and mosquito-borne disease control must perforce be driven to biological methods, even when funds for chemical methods of control are available. Engineering methods will, of course, remain but these are not more than specialised methods of biological control achieved usually at a cost only bearable in exceptional conditions."

9

Resistance to Insecticides

One of the major causes for setbacks in the global programmes of malaria eradication is the development of resistance among vector anophelines to the commonly used synthetic insecticides such as DDT, HCH, dieldrin and even to malathion. Resistance has been reported in malaria vectors in some 62 countries out of 107 where malaria transmission occurs or had previously occurred. The phenomenon is global as it has been found in all continents.

According to WHO Technical Report Series, 585 (1976), in 1975 a total of 42 anopheline species was reported to be resistant in the world. All 42 were resistant to dieldrin/HCH and of them 24 also resistant to DDT. In addition six species had become resistant to malathion. All the species resistant to malathion were also resistant to both the other groups of insecticides.

Though 42 species of anophelines had become resistant by 1975, including some major vectors such as *A. albimanus, A. gambiae, A. atroparvus, A. sacharovi, A. hyrcanus, A. messeae, A. culicifacies, A. stephensi,* etc. Several other vectors had not shown any resistance. Among them were: *A. nuneztavori, A. darlingi* and *A. benarocchi* in the Americas, *A. superpictus* and *A. claviger* in Europe, *A. melas, A. merus* and *A. moucheti* in Africa, *A. punctulatus* and *A. kaliensis* in the Pacific Islands and *A. minimus, A. balabacensis, A. maculatus, A. jeyporiensis, A. campestris* and *A. leucosphyrus* in south east Asia and the western Pacific. *A. fluviatilis* was also susceptible except in one or two small localities.

The problem of resistance is a complex one involving mosquito physiology, genetics, adult bionomics, larval ecology and operational techniques.

What is resistance?

According to the WHO Expert Committee on Insecticides (1957) (Technical Report Series, 125) "resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species." Brown and Pal (1971) have further added that the word "resistance has come to be applied to any population, within a species normally susceptible to a given insecticide, that is no longer controlled by the insecticide in the area concerned. In other words, resistance is a developed attribute that has come to characterize an insect population consequent upon continued treatment with the insecticide. By convention the word is not applied to species that are normally resistant in the first place; instead of calling grass-hoppers or boll-weevils resistant to DDT, it is sufficient to state simply that DDT is ineffective against these species."

Resistance is not developed *de novo* in an individual insect or its progeny. The ability to resist is already present in some members of the population and by selection those specimens survive and their progeny multiplies gradually to become a sizeable proportion of the population or even completely to replace those which were originally susceptible. The phenomenon has a strong genetic basis and follows normal mendelian laws of inheritance.

Resistance can be broadly classified as of two kinds:

(a) Physiological resistance.

(b) Behaviouristic resistance.

In addition, there is the phenomenon of excito-repellency which may not be true resistance but a natural habit of individuals escaping prolonged contact with an insecticide.

Physiological resistance

Some individual mosquitoes which survive contact with an insecticide have the physiological mechanism of detoxifying the insecticide absorbed. The mechanisms vary with different types of insecticides. DDT, for instance, is metabolized by certain enzymes (now called the DDT-dehydrochlorinase) to harmless compounds. This finding, based mainly on studies on houseflies, probably applies to mosquitoes also.

The exact mechanism of physiological resistance to cyclodine compounds (HCH, dieldrin, etc.) is not very clear. Generally selection of resistance to one compound of this group induces resistance to others of the same group.

Resistance to organophosphorus compounds (malathion, diazinon, parathion, etc.) is due to detoxification by hydrolysis catalyzed by esterases such as acetylchlorinesterase. In the housefly, parathion and diazinon resistance has been found to be separate from the resistance to malathion because the latter relies on detoxification by carboxylesterase, malathion being the only compound with a molecular structure open to such detoxification (Brown and Pal, 1971).

Cross resistance

It is now well known that when resistance appears to one insecticide due to selection of one or more genes, resistance generally appears also to other insecticides of the same group. But in recent years the subject has been found to be more complex and the patterns of cross resistance will depend on particular genes. In cyclodine compounds, however, the cross-resistance phenomenon is very marked.

DDT resistance in many species of mosquitoes, like *A. sundaicus, A. stephensi, Aedes aegypti,* etc. does not involve resistance to gamma HCH or dieldrin. Conversely dieldrin resistance of *A. gambiae, A. quadrimaculatus* (also *A. culicifacies* in Thane District in 1958), and HCH resistance in *Culex fatigans* did not involve DDT resistance. Cross resistance between chlorinated hydrocarbons and organophosphorus compounds also does not generally occur. However, under certain experimental conditions, instances of such cross resistance have been noticed. They

are often modified by genetic factors, such as low frequency of genes for a particular resistance. HCH/dieldrin resistance and DDT resistance appear independently. In India, for example, high levels of resistance to DDT and dieldrin and malathion which have been found in Gujarat and Maharashtra States, in *A. culicifacies* appeared one after another as the insecticides were used. In the laboratory it has been shown that cross-resistance occurs with malathion on the one hand and to fenitrothion, etc. on the other hand. However, in many cases populations which are resistant to one type of insecticide, tend to become resistant to other types, more rapidly than when the insecticides are used the first time.

In a few small scale field experiments, fenitrothion (an OP compound) has been used to combat *A. culicifacies* already resistant to DDT and HCH. Bhatia *et al.* (1969) and Bhatnagar *et al.* (1974) working in Maharashtra State and Rajasthan respectively have found it to be a useful substitute for DDT and HCH, but Bhatnagar and Wattal (1979) feel that the development of resistance to fenitrothion also would rapidly occur as was found with *A. albimanus* in Central America. There are unpublished reports that *A. stephensi* in Southern Iran, where it has a high level of resistance to DDT, dieldrin and malathion, has a potential for a moderate level of resistance to fenitrothion also.

It is interesting to note that no resistance has yet been detected in any mosquito against natural pyrethrum extracts. But there is some evidence that resistance may occur to synthetic pyrethrins.

Among anophelines which have developed resistance to OP compounds, carbamates or pyrethroids a few are shown in Table 10.

Table 10. Resistance to OP compounds, carbamates and pyrethroids

	Malathion	Fenthion	Fenitrothion	Parathion	Temephos	Chlorophyrilos	Bromophos	Propozur	Bioresmithon
A. albimanus	×	×	×	×	×	×		×	
A. sacharovi	×	×	×	×			×	×	
A. hyrcanus			×	×			×		
A. sinensis		×	×	×					
A. culicifacies		×							
A. messae		×	×						

From WHO Technical Report Series 585 (1976).

Note: *A. culicifacies* has become resistant to malathion in parts of India.

The mechanism of physiological resistance and cross-resistance is still a developing subject. Though hundreds of studies have been made and the results published, the explanations have not yet been complete or satisfactory. Most of the studies have been made on house-flies and only a few on mosquitoes. It would not be safe to generalize as to the physiological mechanisms applicable to all groups of insects.

Behaviouristic resistance

Behaviouristic resistance is a convenient term used to explain several types of variation in responses to the insecticide, particularly to DDT. It denotes an increased evidence of an escaping ability. One can presume that in mosquito populations there are individuals which have a habit of leaving dwellings soon after feeding. Such individuals are apt to receive sublethal doses of DDT and therefore likely to survive. The survivors may in due course become the main components of the population. In such cases there is no physiological effect or detoxification of the insecticide at all, but conversion of a population with a few individuals escaping to one in which the majority escapes from getting the lethal dose. A well known example of behaviouristic resistance has been noted in the populations of *A. albimanus* in Panama, first reported by Trapido (1954); subsequent examples are of a *A. sundaicus* in Java and *A. atroparvus* in the laboratory. No such phenomena has been recorded in populations of Indian anophelines. However, it is still to be decided whether the present pronounced exophilic behaviour of *A. philippinensis* is Assam/Meghalaya, where formerly it was being collected easily in dwellings, is a true behaviouristic change or merely an influence of the insecticides still being used.

Vigour-tolerance

Vigour-tolerance is a term which has been used to describe the ability of an insect to overcome the effect of the insecticide rather because of the extra vigour of the individual or the strain, rather than from any specific mechanism. Perhaps, such individuals or strains have increased weight or improved biochemical conditions and can withstand contact with the insecticides. The phenomenon is distinct from simple physiological resistance. Though some work has been done which indicates the existence of some genetic basis, it is difficult to distinguish vigour-tolerance from true specific resistance in the field. Brown and Pal (1971) have indicated that true resistance can be distinguished from tolerance where the median lethal dose has a 10-fold increase in mosquito larvae and a 4-fold increase in adults.

Genetics of resistance

Resistance is controlled by specific genes which regulate the appropriate physiological or behaviouristic mechanisms.

DDT resistance is usually recessive in character, dieldrin resistance is either recessive or dominant and OP and carbamate resistance is without exception dominant. DDT resistance can occasionally be intermediate and even dominant.

A single gene for dieldrin resistance has been discovered in several species of

anophelines. In nearly all cases the dieldrin resistance in the hybrids were intermediate in susceptibility tests.

A single gene for DDT resistance has been detected in five species of anophelines. Unlike in dieldrin resistance, clear separation of the phenotypes is not possible with DDT resistance. In most species DDT resistance is recessive and in a few others it is dominant. Even differences may be found in strains of the same species and the two types may co-exist in the same locality. Further, modifying alleles have been found for DDT resistance in some species.

Resistance to OP compounds has also been found to be monofactorial in the housefly, *Culex tarsalis*, and in the African blow fly, *Chrysoma putoria*. There are two types of resistance in the housefly, one to parathion and diazinon and another specifically to malathion. Little work has been done on anophelines.

No resistance to carbamate compounds had been developed in insect vectors till 1969. The subject has undergone rapid development since then. In 1971 Brown and Pal gave instances of resistance to carbamates in *Musca* and *Drosophila*, both dominant. A few mosquitoes have also shown resistance to carbamates.

"It is now fairly well established that single genes are responsible for most cases of dieldrin resistance and the major genes (oligogenes) with ancillary or modifying genes are involved in DDT resistance. Both conditions may pertain to organophosphate and carbamate resistance" (Davidson, 1972). Davidson has further explained that basically the single gene inheritance may reflect either an almost complete dominance, an intermediate condition, or virtual recessiveness.

Davidson has summarized the features of the three types of resistance and has given examples.

Dominant resistance:

Parents	RR	X		ss	
F_1		Rs	X		Rs
F_2	RR	Rs	sR		ss

3 : 1 ratio of resistant to susceptible individuals in the F_2 generation.
Examples: Dieldrin resistance in species *A* and *B* of *gambiae* complex.

Intermediate resistance:

Parents	RR	x	SS	
F_1		RS	x	RS
F_2	RR	RS	SR	SS

1 : 2 : 1 ratio of resistant, intermediate and susceptible individuals in F_2 generation.

Examples: Dieldrin resistance in several species of anophelines and O.P. resistance in anophelines.

Recessive resistance:

Parents	rr	x	SS	
		|		
F_1		rS	x	rS
			|	
F_2	|	|	|	|
	rr	rS	Sr	SS

1 : 3 ratio of resistant to susceptible individuals in F_2.

Examples: DDT resistance in several anophelines.

Note: This type of resistance does not appear to be present to the other types of insecticides.

There are many genetic aspects of resistance which are beyond the scope of this book. But it is worth noting that the ease and rapidity or the slowness of development of resistance in natural populations is often controlled by the recessive, dominant and intermediate types of inheritance. For example, *A. culicifacies* took a long time (over 12 years) to develop measurable loss of susceptibility to DDT while the same species in Thane District, Maharashtra, developed very high resistance to dieldrin with only two applications of the insecticide (Ramachandra Rao and Bhatia, 1957; Patel *et al.*, 1958). Why resistance has developed in some locations or regions and not in others is a subject of extreme interest. Dr B.A. Rao has expressed the view that because DDT had not been used as a larvicide, the speed of development of resistance was low. However, DDT had till recently been used as an agricultural pesticide and what effect it had cannot be estimated.

This rather brief discussion of the fundamental aspects of insecticidal resistance has been presented solely to draw the attention of malaria field entomologists to the basic principles. The literature on resistance is very vast and every year new information is being collected on the genetic, physiological, ecological and even taxonomic aspects of resistance. Much more information is available on other insects than on mosquitoes. The book *Insecticide Resistance in Arthropods* by Dr. A.W.A. Brown and Dr. R. Pal still is the most comprehensive on the subject. It is supplemented by the publications of the WHO. Any further discussions on the basic principles of resistance are beyond the scope of this book which is primarily to deal with Indian anophelines.

Very up-to-date and some new information on insecticide resistance has been provided by the proceedings of the XVI International Congress of Entomology edited by Pal *et al.* (1981).

History of Development of Resistance in India

Soon after the extensive use of synthetic insecticides in public health programmes after the World War II, resistance in insects of public health importance became

evident. In 1946 DDT resistance was first discovered in the housefly from Sweden (Weismann, 1947). Later in 1951 DDT resistance in *A. sacharovi* (Lividas and Georgpoulos, 1953) in Greece was recorded. Dieldrin resistance was discovered in *A. gambiae* in Northern Nigeria.

In India the first report of appearance of DDT resistance in mosquitoes was that of *C. fatigans* in a village near Delhi in 1952. (Pal *et al.*, 1952).*

Among the Indian anopheline mosquitoes, *A. subpictus* was reported to be resistant to DDT in Jwalheri Village (Sharma and Krishnamurthy, 1957). This village had been regularly sprayed with DDT since 1948. Among the vectors, *A. stephensi* was the first to be noticed to have developed resistance in 1955 to DDT (Rajagopalan *et al.*, 1956) in Erode Town, Tamil Nadu.

While DDT resistance was first noticed in 1959 in Gujarat State (former Bombay State) in *A. culicifacies* (Rahman *et al.*, 1959) dieldrin resistance in that species was first noticed in Thane District of Bombay State in 1958. HCH resistance appears to have been first reported from Orissa (Birbatti) in *A. aconitus* and *A. nigerrimus* (Das, 1966). It is worth pointing out here that DDT had been used extensively in the country since 1946 and HCH has been used in limited areas commencing from 1947. Malathion came to be used on large scale in Maharashtra from 1969, though preliminary tests had been made in 1965.

There is no well documented evidence of reversal of DDT resistance under field conditions in India, though a few reports in respect of *A. culicifacies* are mentioned. Reversal of resistance presupposes existence of favourable conditions, particularly that it should have appeared in a small focus surrounded by large areas in which the insect was still susceptible. In such a case rapid infiltration of individuals from the periphery can restore susceptibility. It may, however, be noted that withdrawal of DDT for several years now does not seem to have changed the resistance status of field populations of *A. culicifacies* to any appreciable extent.

An instance of several of resistance to insecticides in the field has been provided by the observations made by the Maharashtra workers (Patel *et al.*, 1958) in Thane District. Following the rapid development of resistance to dieldrin, a severe epidemic of malaria occurred in 1958. Dieldrin was immediately replaced by DDT resulting in very good control. Bhatia and Deobhankar (1963) continued to monitor dieldrin resistance and by 1962 found that in the same area of Thane District, *A. culicifacies* had reverted to normal susceptibility. This was possible because the area in which resistance had developed was very small. It was surrounded by vast areas in which dieldrin or HCH had not been used.

In Bengal also LC_{50}'s for DDT were very low among the two vectors, *A. philippinensis* and *A. sundaicus* in 1958.

*The present author had noticed an almost total ineffectiveness in *Culex fatigans* of indoor sprays of DDT at 56 mgm/sq. foot in Dharwar District, in 1947 itself, hardly after two rounds of spraying. In his view *C. fatigans* has been insensitive to DDT naturally or could develop resistance extremely rapidly. The clamour of people that DDT was ineffective against the nuisance mosquito was so great even in 1947 that the author had to plead with the people to remember that the programme was for malaria control and not for mosquito nuisance control.

Summarizing the then available data, Pal (1958) concluded that except for *A. stephensi*, the vectors of malaria had remained susceptible to DDT and HCH. Higher tolerance to DDT had been observed in *A. stephensi* from Erode (Tamil Nadu) and dieldrin resistance in *A. culicifacies* in Thane, Maharashtra, as stated above. But the position has changed considerably since then.

In order to monitor continuously the susceptibility status of malaria vectors, susceptibility tests have been undertaken on a routine basis under the National Malaria Eradication Programme. The information thus gathered has shown that among the vectors the following have shown resistance to one or another insecticide. *A. culicifacies, A. stephensi, A. annularis, A. philippinensis* and *A. splendidus.*

The resistance shown by *A. annularis* and *A. philippinensis* is restricted to very small areas in the country. Resistance in *A. fluviatilis* in a small locality (in Maharashtra/Karnataka) has yet to be substantiated; otherwise the species is quite susceptible in all other areas. For detailed earlier reports see Das (1966) and Raghavan *et al.* (1967). Wattal *et al.* (1981) feel that the use of the common insecticides in agriculture has been responsible for the development of resistance.

The species of anophelines (Bhatnagar and Wattal,1979) showing insecticide resistance during 1977 were:

	DDT	Dieldrin/HCH	Organophosphorus compounds (malathion)
A. culicifacies	×	×	×
A. stephensi	×	×	×
A. fluviatilis	×		
A. subpictus	×	×	
A. annularis	×		
A. splendidus		×	
A. philippinensis			

Present Status of Resistance in Indian Anophelines (1979)

(A) Vectors

A. culicifacies

This species remained susceptible to DDT during the first 10-11 years of widespread use of the insecticide (Ramachandra Rao and Bhatia, 1957; Sharma and Krishnamurty, 1957; Bhatia, Deobhankar and Vittal, 1958; Pal, 1958). It was thought that *A. culicifacies* was lacking the gene for DDT resistance. Ramachandra Rao and Bhatia (1957) who studied the susceptibility of the species in relation to the length of years of the exposure to DDT drew a correlation curve which showed a gradual loss of susceptibility, but not to that degree which may be classified as resistance. By 1959, however, resistance to DDT was observed in two localities of Panchmahals District of Gujarat (Rahman, Roy and Singh, 1959). This was further confirmed by Luen and Shalaby (1962) who found the LC_{50} to exceed 4 per cent in the same area (Panchmahals District) in 1961. Since then reports of resistance to DDT have appeared in various States such as Gujarat, Maharashtra, Rajasthan, Uttar

Pradesh, Madhya Pradesh, Karnataka and Tamil Nadu (Raghavan *et al.*, 1967; Bhatnagar and Wattal, 1979).

The distribution of insecticide resistance in *A. culicifacies* can be gauged from the following table (Table 11) taken from Bhatnagar and Wattal (1979) showing results upto the end of 1977.

Table 11. Development of insecticide resistance in NMEP Units (From Bhatnagar and Wattal, 1979)

State	*No. of units under National Malaria Eradication Programme in which resistance has been found.*		
	DDT	HCH (BHC) & DDT	DDT, HCH & Malathion
Maharashtra	20	16	1
Gujarat	17	13	1
Madhya Pradesh	27	2	—
Uttar Pradesh	18	—	—
Rajasthan	8	1	—
Punjab	8	1	—
Karnataka	7	—	—
Bihar	4	—	—
Haryana	1	—	—
Tamil Nadu	2	—	—
Orissa	1	—	—
Jammu & Kashmir	1	—	—
Total	**114**	**33**	**—2**

In addition, DDT and HCH resistance has been found in Delhi State also.

Unfortunately the number of units in which the species was still susceptible is not recorded. While the above table gives an indication of the wide distribution of resistance in the country it may not adequately reflect the prevalence of resistant populations in each State because except for a few States, such as Maharashtra, Gujarat, Delhi and Madhya Pradesh, susceptibility tests have not been carried out extensively in many of the units in other States. Perhaps if such tests had been carried out in all units, the picture might have been somewhat different.

Dieldrin resistance appeared in *A. culicifacies* in 1958 after only two rounds of dieldrin spraying in Thane District of Maharashtra. This resistance was accompanied by an outbreak of malaria in the area (Patel *et al.*, 1958) which was controlled by the reintroduction of DDT which was still effective as stated above. The LC_{50} for dieldrin was 3.1 per cent, about six times more than that recorded for the species at that time in India (Bhatia *et al.*, 1958).

HCH resistance appeared in Maharashtra after its use for a period of two years. The same pattern followed in Gujarat and recently in Andhra Pradesh. Double resistance to DDT and HCH has been reported from Gujarat and Maharashtra by Sharma and Samnotra (1962). Bhatnagar and Wattal (*loc. cit.*) recorded double resistance in five States.

Resistance to malathion (for the first time in India) in *A. culicifacies* was found

by Rajagopal (1973) in Gujarat. There was a four fold increase in LC_{50}. The area had received six rounds of Malathion spray from 1970-72 (Rajagopal, 1977). The triple resistance in that area consisting of large areas of four districts, Surat, Bulsar, Thane and Nasik, is posing a serious problem.

A. stephensi

The first evidence of DDT resistance in *A. stephensi* was discovered in 1955 in Erode Town of Tamil Nadu. Here the tests were done on *A. stephensi* larvae (Rajagopalan, Vedamanickam and Ramoo, 1956). Subsequently the resistance to DDT in *A. stephensi* in adults was observed in Salem, Bhavani and Kumarapalyam (Bhombore, Roy and Samson, 1963). DDT resistance was also found in Andhra Pradesh in 1962, at Guntur, Visakhapatnam and Hyderabad City (WHO Expert Committee on Insecticides, 1963; Rao and Sitaraman, 1964). In addition, Karnataka, Maharashtra, Gujarat, Rajasthan, Madhya Pradesh and Tamil Nadu have reported resistance either to DDT or to HCH but double resistance has been reported only from Gujarat and Maharashtra (Sharma and Samnotra *loc. cit.*, 1962). Resistance to HCH has also been recorded from Calcutta and Kotah, Rajasthan (Bhatnagar and Wattal, 1979).

A. annularis

Resistance to DDT in *A. annularis* was first found in a village in the Meerut District of Uttar Pradesh in 1962 (Krishnamurthy and Singh, 1962). Azeez (1964) reported resistance to DDT in a vilalge near Dhanbad, Bihar. In the Panchmahals District of Gujarat State intermediate DDT resistance was observed (Bhatia, Deobhankar and Vittal, *loc. cit.*, 1958). A similar DDT resistance was evident in Udaipur, Rajasthan (WHO Sixth summary of cases of insecticide resistance in anopheline mosquitoes, Geneva; unpublished document quoted by Brown and Pal, 1971). Higher tolerance to DDT in Ratlam, Madhya Pradesh (Madhya Pradesh State Mal. Org., 1965) and resistance to DDT in Tripura State have also been recorded in this species (Krishnamurthy—unpublished document). However, in 1966, Das (1966) had not found any loss of susceptibility to DDT and HCH in this species in Orissa.

A. fluviatilis

Susceptibility tests carried out on *A. fluviatilis* have been very few due to paucity of adults. However, susceptibility tests carried out in 1958 in Panchmahals District showed that the species was susceptible to DDT (Bhatia, Deobhankar and Vittal *loc. cit.*, 1958). It was also found that *A. fluviatilis* was susceptible to DDT near Dhanbad, Bihar (Azeez, *loc. cit.*, 1964). The species is still regarded to be quite susceptible, but DDT resistance was reported from Pandharpur, Maharashtra State, with DDT tolerance in two other localities and intermediate DDT resistance from Sakleshpur and Bijapur in Karnataka (unpublished NMEP records). These reports need confirmation.

A. sundaicus

So far there has been no report of resistance in *A. sundaicus* to DDT or HCH in India, though in some of the countries of the Southeast Asian region (Indonesia) it is resistant to DDT and dieldrin. In fact the current populations of *A. sundaicus* in India are so low that enough adults are not available for tests. The species inspite was found highly susceptible to DDT in the Andamans in 1979 (Kalra, 1981).

A. philippinensis

A. philippinensis has so far been found resistant to DDT only in Assam (NICD Report, 1970—quoted by Krishnamurthy).

Nawab Singh and Chakravorty (1979) tested in 1972-1973 the susceptibility of *A. philippinensis* to DDT in a few areas in the border of India and Bangladesh, in Assam, Meghalaya and Tripura, and found that the species was still highly susceptible. All tests with 4.0 per cent papers give 100 per cent mortality. Even in areas which in 1970 had shown some degree of resistance to DDT showed no resistance in 1972. The species is also susceptible to BHC.

A. minimus

After the countrywide spraying of insecticide (DDT) under NMEP *A. minimus* has virtually disappeared and it has become very hard to collect any adult specimen in indoor or outdoor shelters. Consequently the susceptibility tests done in the country are very few. It is, however, regarded to be still susceptible.

(B) Non Vectors:

A. aconitus

This species has been found susceptible to DDT, dieldrin and HCH in Calcutta and its suburbs. However, in Birbatti in Orissa, *A. aconitus* was tolerant to DDT, but definitely resistant to dieldrin and HCH (Das, 1966). It was three times more resistant to DDT, more than 27 times tolerant to dieldrin and 11 times tolerant to HCH. Owing to rarity of adults enough tests have not been done.

A. barbirostris

In unsprayed localities *A. barbirostris* showed natural tolerance (Pal, 1958). Subsequently some heterogeneity in the composition of the population was noted (Das, 1966).

A. nigerrimus

Das (1966) found in this species in Orissa, that the response to DDT was highly heterogenous thereby indicating higher tolerance to DDT. It was also shown that there had been a selection for resistance to dieldrin.

A. subpictus

A. subpictus was first found to be resistant to DDT in Jwalaheri, Delhi State in the year 1955. The village was under DDT spray from 1948 (Sharma and Krishnamurthy, 1957). DDT resistance has since developed in Delhi, Pune, Panchmahals and U.P. However, it is believed that resistance to DDT is quite widespread in the country because of the large numbers collected in sprayed structures.

Pal and Bhalla (1959) found DDT resistance and dieldrin resistance in West Bengal.

A. splendidus

This species has been found to have developed dieldrin resistance at Singhbhum, Bihar in 1957-58 (WHO Seventh Summary of cases of insecticide resistance in anopheline mosquitoes, Geneva), (unpublished document quoted by Brown and Pal (1971).

A. tessellatus

Kalra (1981) found this species be susceptible to DDT in the Andamans during 1979-80.

Resistance of Indian anophelines in neighbouring countries

Some of the vector species of India have also been found resistant to DDT and/or HCH in the neighbouring countries (WHO, TRS, 585, 1976). They are:

A. culicifacies	... Afghanistan, Iran, Pakistan, Nepal, Sri Lanka.
A. stephensi	... Afghanistan, Iran, Iraq, Pakistan, Saudi Arabia.
A. fluviatilis	... Saudi Arabia.
A. annularis	... Nepal, Bangladesh, Indonesia.
A. aconitus	... Indonesia.
A. sundaicus	... Indonesia, Malaysia.
A. minimus	... Indonesia.
A. philippinensis	... Malaysia.

The non-vectors which have similarly become resistant in the neighbouring countries are:

A. pulcherrimus	... Afghanistan, Iraq, Saudi Arabia, Syria.
A. multicolor	... Saudi Arabia.
A. subpictus	... Pakistan, Nepal, Sri Lanka, Indonesia, Bangladesh.
A. sinensis	... Japan and Korea.
A. barbirostris	... Indonesia.
A. vagus	... Bangladesh, Vietnam, Indonesia, Philippines, Malaysia.

In brief, it may be stated that resistance to DDT and BHC is widespread among the two major vectors, *A. culicifacies* and *A. stephensi*, in the country, but other vectors, particularly *A. fluviatilis* and *A. philippinensis*, are still largely susceptible. Some vectors like *A. minimus* and *A. sundaicus* have greatly dwindled in their occurrence, and are still susceptible. Unfortunately there are vast areas where tests have not been made and therefore a complete map of resistance as well as susceptibility cannot be made. Bhatnagar and Wattal (1979) seemed to think that effective use of DDT or BHC was still possible in many parts of the country.

Malathion resistance has already shown its ugly head on the Maharashtra and Gujarat border. How the vectors will react when the insecticide it used more widely cannot be predicted.

Insecticidal resistance is a subject to which Indian entomologists have to give expert attention, in all its aspects, viz. physiology, biochemistry, genetics and behaviour. Unfortunately all that they have done till now is to carry out routine and sometimes specialized susceptibility tests.

In the above account reference to literature have been made only of a few comprehensive or major articles. Many publications referring to susceptibility tests made in local situations exist. Very valuable as they are, it has not been found possible to refer to them because of lack of space.

Detection and Measurement of Insecticide Resistance in Anophelines

The development of resistance to insecticide generally comes to the notice of malariologists when the adult anophelines resting inside sprayed structures rapidly increase in numbers after the spraying necessitating further spraying at shorter intervals. The numbers in the sprayed structures reach those in unsprayed structures much sooner than normally. Occurrence of new malaria cases even when the spray coverage has been satisfactory also gives an indication that the insecticide has not been effective. A series of tests on the susceptibility of the vector to insecticides carried out with the help of the special kits designed by the World Health Organisation can confirm the development of resistance. Such tests should become a routine feature of all large-scale programmes of vector control by use of insecticides and should be carried out concurrently with the spraying operations in a good number of representative localities so that resistance may be detected even when it is incipient and suitable steps taken to overcome it. The methods of using the standard test kits and of interpreting the data have been fully described by Brown and Pal (1971) and in the WHO *Manual on Practical Entomology in Malaria, Part II* (1975). Workers should refer to these publications for detailed instructions. Tests are available both for larvae and adult mosquitoes.

Test on Adults

The essential features of the standard WHO tests are:

(a) Collection of adult mosquitoes from the field, keeping note whether they are from sprayed or unsprayed structures, dates of spraying, gonotrophic condi-

tion etc. Laboratory bred adults, reared from larvae collected in nature, may also be used.

(b) Introducing 15-25 females in the same physiological condition in the exposure chamber of the test kit, which is lined by an absorbant paper which has been impregnated with a known concentration of the insecticide dissolved in a solvent oil, and exposing the mosquitoes for a period of one, sometimes two hours, in the chambers.

(c) Transferring the adults to clean recovery chambers lined by unimpregnated papers and keeping them for 24 hours at the end of which noting down the number dead.

(d) Computing the mortalities among the mosquitoes exposed to the insecticide and in the controls and calculating the adjusted mortalities by applying Abbott's formula.

(e) Plotting the data on log probit graph papers for obtaining regression lines and determining the dosage-mortality relationship or the diagnostic concentration.

Test on Larvae

The essential features of the test are to expose late 3rd or early 4th instar larvae to various concentrations of the insecticide in ethanol-water solutions for a definite period of time and to determine mortalities after a fixed holding period. In the WHO standard method used for routine tests, the larvae are exposed for a period of 24 hours and mortalities determined immediately after exposure. In Elliot's method, used to isolate resistant individuals, the exposure period is one hour and holding period 5 hours after which the mortalities are determined.

Measurement of Insecticide Resistance

Base line data for each species and for each locality or region are collected preferably in localities in which the particular insecticide had not been used so far. Such localities are, however, difficult to find now a days because of the extensive use of insecticides in agriculture. Preliminary tests should, however, be done in areas which are to be taken up for insecticidal treatment. Later on routine susceptibility tests are to be carried out concurrently with the programme of insecticidal applications.

Two objectives are usually aimed at: 1) Delineation of dosage-mortality curves or regression lines from which the median lethal concentrations (or dosage) can be determined and 2) Determination of the diagnostic concentration.

(1) Dosage mortality regression lines

Median lethal concentration or dose is the concentration of an insecticide in the

impregnated paper or solution at which 50% of the exposed insects die after the standard period of exposure and recovery. It is denoted as LC_{50} or LD_{50}. Similarly concentrations that kill 40%, 60% or 100% are denoted as LC_{40}, LC_{60} and LC_{100}. To obtain meaningful results the mosquitoes should be exposed to at least 4 concentrations, one of which gives complete kill and at least one gives less than 50% kill. These mortalities are then plotted on log-probit papers and percentage mortalities are read off from the regression lines. With experience it has been found that with several species of mosquitoes with respect to organochlorine, organophosphate and carbamate insecticides, the time is interchangeable with concentration i.e. LT_{50} with LC_{50}. It is, however, necessary that for lethal time, measurements insects are neither exposed for long intervals i.e. over 8 hours or short periods less than 15 min.

Generally when the dose mortality data are directly plotted on the log-probit papers to get regression lines, a steep line indicates homogeneous populations. The slope values of the regression lines also mean the degree of homogeneity of the populations. A susceptible line occurs at the low dosage levels (corresponding to base line date as shown in Figure 6, line 1). If the regression lines shift towards higher dosages and remain parallel with the base line, this indicates an increase in tolerance only. If after exposure for 2 hours a straight regression line parallel to the base line is obtained, this indicates vigour tolerance (line 2 fig. 6). If the line forms a plateau where the increase in dosage produce no increase in kill, it indicates the presence of true resistant individuals in the population. Therefore flattening of tip of the regression lines are due to incipient resistance. Resistant population would show no kill at the dose levels shown in Fig. 6.

It may be pointed out that lines are likely to be affected by many factors including the number of genes involved, whether dominant or recessive, temperature, physiological conditions etc. Therefore they should be interpreted with caution. As a guideline for mosquito larvae a 10-fold increase in LC_{50} is necessary to indicate resistance, and for adults 4-fold increase is sufficient. In cases where this increase is less than the above values, word tolerance may be used (Brown and Pal, 1971).

(2) Diagnostic Concentrations

After the base line data have been collected for a given species of mosquito and a given insecticide, it would not be necessary to carry out tests with the whole range of concentrations to detect resistance. A single dose known as the diagnostic concentration (formerly known as discriminatory dose) is employed. This is the concentration which gives a complete kill. (On log-probit-papers it is the one which gives a 99.9% mortality). W.H.O. advises that as a 'rule of thumb' double this concentration should be used to reduce the chances if any ambiguity from exceptional susceptible survivors. The presence of any survivors at this dose would indicate the occurrence of resistance. The tentative diagnostic concentrations for anophelines

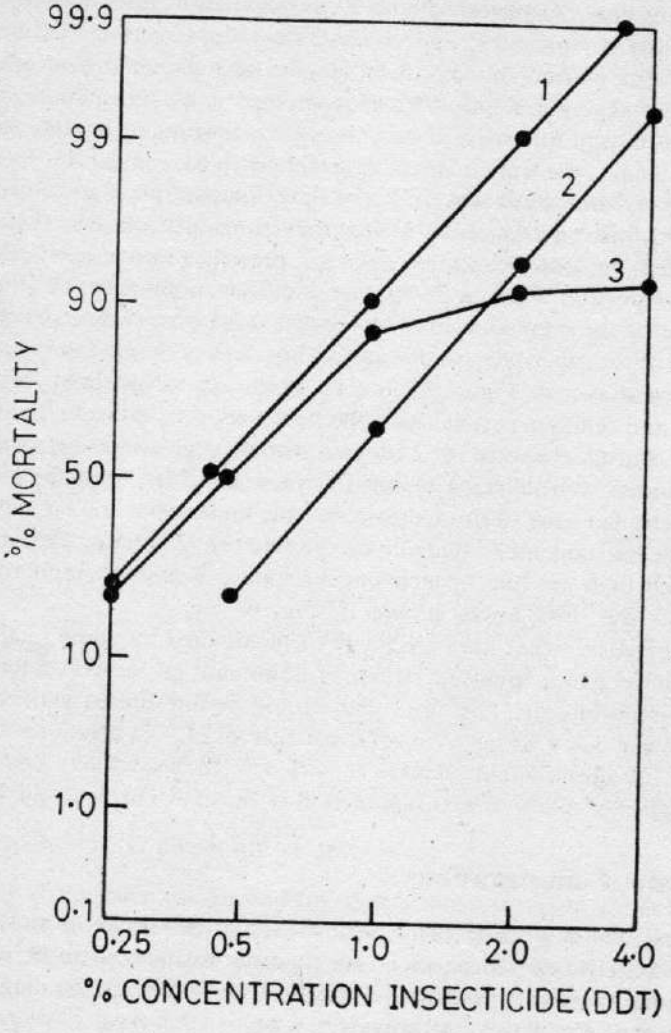


Fig. 6 : Showing the response of insects to insecticide concentrations; Line 1 susceptible, Line 2 vigour tolerance and Line 3 incipient resistance; flattering shows the presence of resistant individuals.

recommended for different insecticides by W.H.O. are given below:

Insecticide	WHO adult test	WHO larval test (24 Hour)
DDT	4.0% for 1 hour	2.5 ppm
HCH	—	0.5 ppm
dieldrin*	0.4% for 1 hour	0.1 ppm
malathion	5.0% for 1 hour	3.125 ppm
fenitrothion	1% for 2 hours	0.125 ppm
fenthion	2.5% for 1 hour	0.05 ppm
parathion	—	0.125 ppm
Abate (temephos)	—	0.25 ppm
Dursban (Chlorpyrifos)	—	0.025 ppm
Propoxur	0.1% for 1 hour	—

*In partially dominant dieldrin resistance in adult mosquitoes, a discriminating or diagnostic concentration of 4% dieldrin for 2 hours will kill heterozygotes but not homozygous resistant individuals.

WHO (Tech. Report Ser. 655, 1980) has recommended a suitable policy for the detection of vector resistance in a malaria control programme based on indoor residual spraying of the insecticides. The diagnostic concentration test should be applied as widely and consistently as possible. This is applicable to both adults and larvae. One in every 10 or one in every 100 villages should be investigated on annual basis. Consideration should however be given to the number of vector species involved and the degree of ecological and socio-economic heterogeneity of the control area. The whole problem of detecting and measuring resistance, though it has been simplified by the use of standard test kits, needs very critical appraisal and shrewd judgement.

Overcoming Resistance

Much thought has been given to the problem of overcoming resistance in nature. Apart from changing over to another group of insecticides no other satisfactory method has yet been discovered. Several new insecticides which hold good promise have been developed but many of them are still very expensive and the safety for humans and domestic animals have to be fully confirmed. A deeper knowledge of the physiology and the genetics of resistance may in future years help in preventing and even reversing the trends towards resistance. Some of the other methods sometimes thought of are: Mosaic application of insecticides, mixture of insecticides, improving the formulations, spraying in selected places, using negatively correlated insecticides, diluting the resistant populations by introducing susceptible genotypes, and integrated control, etc. Though some of them have given hopeful results in laboratory or small field experiments, they have not been of much avail in large scale work in the field. Intensified research would certainly help in solving the problem if pursued with complete objectivity and vigour.

10

Some Long Term Changes in Anopheles Populations as a Result of the Use of Residual Insecticides

The use of DDT and other residual insecticides on a wide and massive scale has brought about certain changes in the species composition and also perhaps certain changes in the bionomics of a few species. A few important among them may be mentioned:

1. *A. minimus* which was the most important vector of malaria in the Himalayan foothills and in eastern India has practically disappeared. All recent surveys have yielded either no specimens or only negligible numbers. However, the species persists in Thailand where it is still a vector along with *A. balabacensis*. In Burma also it appears that its prevalence has been greatly reduced. It appears that in Thailand the species, though abundant, does not rest in houses or cattle sheds. They are collected in large numbers in night biting collections. It has been postulated that there may be two races and the use of DDT has wiped out the house frequenting race and only the exophilic race has survived. This needs further field studies.

2. *A. sundaicus* has made a dramatic withdrawal from the Orissa and Andhra Pradesh coasts and even in Bengal it occurs in negligible numbers. In fact, the 1976 report of the N.M.E.P. indicates that a few specimens were persisting in the 24 Parganas District in W. Bengal, but the number was so small that enough numbers were not available to carry out insecticide susceptibility tests. It however persists in the Andamans.

3. *A. philippinensis,* which has long been regarded as a major vector in the Deltaic areas of Bengal and Assam seems to have undergone a great reduction in Bengal where very few specimens have been collected in houses in recent years. In Meghalaya also it is now-a-days extremely scarce inside houses and cattle sheds, but very large numbers can be collected in outdoor or indoor biting collections at night. There is urgent need to make night collections on man and animals both in Assam and Bengal, as has been done in Meghalaya. Apparently, the species has undergone a change in its resting habits. Is the scarcity in houses a true behaviouristic change or merely an avoidance of the insecticide treated premises? There is a great scope for studies on this subject in Bengal and Assam.

4. *A. fluviatilis* of the Western Ghats, which was found more in houses than in cattlesheds (ratio 9:1) during the pre-DDT era, is now reported to be more common in cattlesheds, though the total numbers have much dwindled. As there is very little locally transmitted malaria in such district as North Kanara, Shimoga, Kadur and Hassan in Karnataka and in Kerala where it was formerly an efficient vector there is presumptive evidence that the man-biting habit has been replaced by cattle biting habit. Is it possible that the man-biting strain, for the existence of which there was strong evidence in former times, has been completely knocked out as in the case of *A. minimus*. *A. fluviatilis*, however, persists in small numbers in Thane-Surat area of Maharashtra and neighbouring districts of Gujarat where the species was the major vector. It has given place to *A. culicifacies*.

5. The major vector in Thane District of Maharashtra prior to the use of DDT in 1944-1948 was *A. fluviatilis*. Many infections had been detected in this species in Salsette Island near Bombay. *A. fluviatilis* has also been found infected in the neighbouring districts of the Western Ghats such as Kolaba, Dangs and Nasik. *A. culicifacies* was present and had also been found infected at a lower rate. All current reports since 1958 bring out the importance of *A. culicifacies* as the major vector. It has become resistant to DDT, HCH and also to malathion and occurs in enormous numbers and is the cause of persistent malaria. What has happened to *A. fluviatilis?* It occurs in such small numbers that the malariologists do not mention it at all in discussing the problems of malaria persistence in this area. There is no evidence that it has become resistant or even tolerant to DDT. Is it probable that it has lost its effectiveness as a vector? If it is still a vector, it is undoubtedly a vector of very low status completely overshadowed by *A. culicifacies*. Here is yet another probable instance of change in species composition and vectorial status in the course of years. It merits deep studies. What could be the factors for the increased prevalence of *A. culicifacies*?

6. Very few comparative observations on total anopheline populations in pre and post-DDT era are available for a meaningful study of long term effects on population. of anophelines. However, very recently Rajagopalan *et al.* (1979) and Chandrahas *et al.* (1979) have made some comparative studies on the mosquito fauna of Pattukkottai Taluk, Tamil Nadu, because it was an area in which fairly reliable data were available for the pre-DDT era. They have found that while *A. subpictus* has continued to occur in very large numbers, perhaps even in larger densities than before, there has been a reduction in the numbers of *A. culicifacies* compared to the data collected in 1938-40 by Russell and Ramachandra Rao (1941a). The proportions of *A. culicifacies*, *A. subpictus* and *A. vagus* collected by them as well as by Rajagopalan *et al.*, (1979) and Chandrahas *et al.*, (1979) are given below:—

	Russell and Ramachandra Rao (1941a)	Rajagopalan *et al.* (1979) Non-transmission season	Chandrahas *et al.* (1979) Transmission season
A. culicifacies ♀	2560	333	1040
A. subpictus ♀	2474	4948	18362
A. vagus ♀	2819	760	48

It may be seen that the proportions of the species have greatly altered and *A. subpictus* is much more prevalent than before. But this may not necessarily mean that these are actual reduction in densities of *A. culicifacies*. Therefore, the following comparative data provided by Rajagopalan *et al.* and Chandrahas *et al.* are interesting:—

Per-man-hour figures

	Russell and Ramachandra Rao 1937-40	Rajagopalan *et al.* (1979) Jan—Mar 78	Chandrahas *et al.* (1979) Aug—Sept 78
A. culicifacies			
Transmission season	40	—	1.9
Non-transmission season	9	1.1	—

The above data showed that a marked reduction in the population of *A. culicifacies* had actually occurred. The reasons are not clear, but Rajagopalan *et al.* point out that the following major changes have occurred in the ecology of the area.

(a) Tremendous increase in acreage under cultivation.
(b) Near total replacement of organic manures by artificial manures.
(c) Many fallow lands have been brought under cultivation under the expansion of the irrigation system.

However, there does not seem to have been much change in the prevalence of species like *A. varuna* and *A. nigerrimus*. It should be noted that there is now no malaria prevalence in Pattukkottai area. It is very desirable that such comparative studies should be made in other parts of India where reliable population data are available for the pre-DDT era.

11

Surveys of Anophelines

Regional surveys provide very useful information on the distribution and prevalence of anopheline species. The results of such surveys offer valuable information on the malaria vectors and their prevalence. Instead of giving district wise distribution lists of anophelines, as done previously by Covell, Puri and others, it has been decided here to give brief summaries of the actual surveys carried out by several workers in each zone. Only some of the important and more comprehensive surveys will be mentioned. One should realize that many of the minor surveys carried out recently by the malaria workers have been incomplete or directed solely to find the vector. Therefore, many of the recent studies of a casual nature are not included. It is hoped that the surveys dealt with in the following pages will provide reliable idea regarding the prevalence of the anophelines and malaria vectors in each zone.

The surveys are grouped together under nine zones of India already identified (Chapter 3).

ZONE I—NORTH WEST AND RAJASTHAN, ETC.

1. Kutch (Now part of Gujarat State)

Afridi *et al.* (1938) carried out a malaria survey of Kutch State in the year 1937 (January and September).

Malaria was prevalent in localized areas only. The anopheline mosquitoes of the following species were collected:

A. annularis
A. barbirostris
A. culicifacies
A. fluviatilis
A. stephensi
A. subpictus
A. turkhudi

A. subpictus was the most predominant species (87.6 per cent) of the total catch while *A. culicifacies* and *A. stephensi* formed 4.1 per cent and 7.2 per cent respectively of the total anophelines collected. *A. fluviatilis* was almost insignificant as only one specimen was collected.

A. stephensi was the only species found infected. Out of 238 of the species dissected, 5 were positive with gut and gland infections. (Total infection rate of about 2.1 per cent).

2. Kaira, Ahmedabad and Panchmahals Districts

The Bombay Malaria Organisation carried out malaria surveys in the districts of

Kaira (during 1947-48), Ahmedabad (during 1947-48) and Panchmahals (during 1948-49) and collected and dissected the following species of anophelines (Viswanathan, 1950) (Table 12 a,b).

Table 12a. Anopheles of Gujarat State

Species	Kaira District	Ahmedabad District	Panchmahals District
A. annularis	1,063	5,477	3,344
A. culicifacies	3,222	1,478	5,632
A. fluviatilis	—	—	213
A. jamesii	—	—	4
A. multicolor	2	—	—
A. pallidus	46	—	3,109
A. pulcherrimus	—	—	3
A. splendidus	—	—	15
A. stephensi	41	165	630
A. subpictus	7,612	20,043	8,464
A. tessellatus	1	—	14
A. varuna	—	—	14
A. vagus	3	1,729	6
A. turkhudi	—	—	45
Total	**11,990**	**28,892**	**21,493**

Table 12b. Dissections of Anophelines in parts of Gujarat State.

	Dissections					
	Kaira District		Ahmedabad District		Panchmahals District	
	No. positive	*No. dissected*	*No. positive*	*No. dissected*	*No. positive*	*No. dissected*
A. annularis	0	255	0	5,497	0	882
A. culicifacies	3 (1 gut 2 glands)	1,343	4 (2 guts 2 glands)	737	0	2,778
A. fluviatilis	—	—	—	—	1 (gland)	124
A. jamesii					0	1
A. pallidus	0	1			1	187
A. pulcherrimus						
A. splendidus						
A. stephensi	0	3	0	52	0	305
A. subpictus	0	35	0	672	0	790
A. turkhudi					0	5
A. vagus			0	824	0	5

A. subpictus was the most predominant species followed by *A. culicifacies* and *A. annularis*. The total absence of *A. jeyporiensis* and *A. nigerrimus* in the indoor collections is noteworthy.

These mosquitoes were almost entirely from rural areas, in which *A. culicifacies* and *A. fluviatilis* are vectors. Panchmahals District is more hilly and more forested than Kaira and Ahmedabad Districts.

3. Broach Town (Gujarat State)

An outbreak of malaria occurred in Broach Town in the year 1963 and 1964. Nair and Samnotra (1967) who investigated the causes of the outbreak, collected the following adult mosquitoes:

A. annularis	*A. stephensi*
A. culicifacies	*A. subpictus*

Two *A. stephensi* out of 87 dissected showed infections (one with sporozoites and the other with oocysts).

In all 832 *A. stephensi* were collected. This species was found in good numbers in the malaria infected areas. It was present both in the cattle sheds and human dwellings.

4. Ahmedabad City

Jaswant Singh and Jacob (1943) carried out a malaria survey of Ahmedabad City from May to November 1941.

They found *A. stephensi* and *A. culicifacies* infected with an infection rate of 1.4 per cent for the former and 0.42 for the latter (one gland infection in 234 specimens dissected).

ZONE II—WESTERN ZONE

1. North Kerala

Covell and Harbhagwan made a survey in Wynaad District (formerly in Madras, now in Kerala State) in 1938 and 1939 (Covell and Harbhagwan, 1939). This district, which nestles in the mountaneous and thickly forested areas in the southern part of the Western Ghats, has long been known to be a highly malarious area. They found 19 species of anophelines (Table 13).

The bulk of *A. fluviatilis* was found in houses and comparatively few in cattle sheds. The scarcity of *A. varuna* is interesting. (Compare with Mathew, 1939).

The most common species found in houses were *A. fluviatilis, A. subpictus, A. vagus* and *A. aconitus*. In cattle sheds, the most common species were *A. jeyporiensis* and *A. aconitus*. While 6,680 *A. fluviatilis* were captured in houses, only 183 were captured in cattle sheds. *A. culicifacies* was comparatively less frequent, only 138 and 176 being collected in houses and cattle sheds respectively.

8,763 anophelines of 15 species were dissected. Out of 3,155 *A. fluviatilis* dissected 467 were gut positives and 224 gland positives (total 592), giving a total infection rate of 8 per cent. Other species were negative. These data should be

Table 13. Anophelines in Kerala State

Species	Human dwellings	Cattle sheds
A. aconitus	350	4,730
A. aitkenii	—	1
A annularis	—	10
A. barbirostris	1	54
A. culicifacies	138	176
A. fluviatilis	6,680	183
A. nigerrimus	29	151
A. jamesii	298	3,191
A. jeyporiensis	178	4,771
A. karwari	1	52
A. leucosphyrus—		3
(*A. balabacensis* or *A. elegans*)	—	
A. majidi	5	120
A. maculatus	—	2
A. pallidus	—	1
A. splendidus	1	105
A. subpictus	980	432
A. tessellatus	17	296
A. vagus	495	1,048
A. varuna	—	1
Total	9,173	15,327

compared with the data of Jaswant Singh and Jacob (1944) and Viswanathan (1950) in North Kanara.

2. South Kerala

In the southern parts of the old Travancore State out of 7,882 female anophelines dissected in two areas during an epidemic in 1935-36, Mathew (1939) made the following observations:—

	Dissected	Gut positives	Gland positives
A. fluviatilis	2,602	—	339 (13%)
A. varuna	429	10 (2.3%)	7 (1.6%)
A. culicifacies	1,131	6 (0.5%)	3 (0.26%)
A. jeyporiensis	3,559	0	0

Others were all negative.

A. fluviatilis was undoubtedly the major vector, but the importance of *A. varuna* was also clearly indicated. *A. culicifacies* was a weak vector, but if the densities were high, it could be a serious vector.

3. Anaimalai Hills—Western Ghats (Tamil Nadu)

Measham and Choudhury (1934) collected and dissected 760 specimens of the following anophelines in an estate in the Anaimalais (Elephant Hills).

A. annularis	(9)
A. barbirostris	(9)
A. culicifacies	(16)
A. fluviatilis	(203)
A. "hyrcanus" var. *nigerrimus*	(94)
A. jamesii	(38)
A. jeyporiensis	(41)
A. maculatus	(48)
A. majidi	(1)
A. pallidus	(21)
A. splendidus	(1)
A. subpictus	(69)
A. tessellatus	(57)
A. vagus	(135)
A. varuna	(19)

The figures in brackets are numbers collected.

Except for *A. fluviatilis*, no other mosquito was found positive. Of the 203 *A. fluviatilis* dissected, 18 infections were found (10 gut, 5 gland and 3 both gut and gland) giving a total infection rate of 8.86 per cent.

4. Nilgiris District, Tamil Nadu

Nilgiris District was very extensively surveyed by Russell & Jacob (1942) between 1937 and 1940. With special reference to the year 1940, they found 23 species of anophelines, *viz:*

A. aconitus
A. aitkenii
A. annandalei
A. annularis
A. barbirostris
A. culicifacies
A. fluviatilis
A. gigas
A. gigas var. *simlensis*
A. hyrcanus var. *nigerrimus*
A. jamesii
A. jeyporiensis
A. karwari
A. leucosphyrus (=*A. balabacensis* or *A. elegans*)
A. lindesayi var. *nilgiricus*
A. maculatus
A. majidi
A. pallidus
A. splendidus
A. subpictus
A. tessellatus
A. vagus
A. varuna

Covell and Puri (1936) had listed 29 species in the entire old State of Madras. Russell and Jacob added two more species, *A. annandalei* var. *interruptus* and *A. gigas* var. *simlensis*, making a total of 31 species. They were unable to collect the following species previously reported, viz. *A. culiciformis, A. insulaeflorum, A. minimus, A. moghulensis, A. philippinensis, A. sintoni, A. stephensi, A. theobaldi* and *A. turkhudi.*

They found a marked difference in the prevalence of species in the western part of Nilgiris exposed to south west monsoon (between June and October) and the eastern part exposed to north east monsoon (between November and January). While 4,182 *A. fluviatilis* were collected in one year in Nilgiris East, only 129 were collected in Nilgiris West. *A. jeyporiensis* was predominant in Nilgiris West and *A. fluviatilis* in Nilgiris East. At the higher altitudes as in Coonoor, *A. fluviatilis* was collected upto an altitude of 6000 feet (1800 metres) above mean sea level. *A. fluviatilis* was the only species found positive for plasmodia.

No. dissected	Total Positive	Gut	Gland
2,580	514 (19.9%)	252 (9.7%)	262 (10.1%)

Positive specimens were found throughout the year except February. In 1,515 dissections of other species, no positives were found.

5a. Shimoga and Hassan District (Karnataka State)

Rao *et al.* (1952) in their survey of anopheline mosquitoes in heavy rainfall areas of Shimoga (1946-47, 1949-51) and Hassan District (1950-52) collected the following 24 species:—

A. aconitus
A. aitkenii
A. annandelei
A. annularis
A. barbirostris
A. culicifacies
A. fluviatilis
A. "hyrcanus" (=*nigerrimus*)
A. insulaeflorum
A. jamesii
A. jeyporiensis var. *candidiensis*
A. karwari
A. leucosphyrus (*elegans?, balabacensis?*)
A. maculatus
A. majidi
A. pallidus
A. philippinensis
A. splendidus
A. stephensi
A. subpictus
A. tessellatus
A. turkhudi
A. vagus
A. varuna

5b. Hassan District, Karnataka State

In Hassan District, a wooded area in the Western Ghats of Karnataka, Brooke Worth (1953) found the following species in one year 1951-52 (Table 14).

The location was at an altitude of just under 800 metres. The numbers of adults and larvae collected have been presented in order to give an indication of their relative abundance in the collections.

A. jeyporiensis was the most abundant of the adults in the high rainfall area of the Western Ghats, as it was in the North Kanara also.

Brooke Worth (*loc. cit.*) has discussed the relationship between the numbers of

Table 14. Anophelines in Hassan District

	Number collected	
	Adults	Larvae
A. aconitus	56	67
A. aitkenii	10	460
A. annularis	400	46
A. barbirostris	95	3,069
A. culicifacies	571	33
A. fluviatilis	116	428
A. nigerrimus	31	2,588
A. insulaeflorum	0	9
A. jamesii	115	536
A. jeyporiensis	3,608	2,064
A. karwari	14	25
A. jeyporiensis var. *candidiensis*	3	0
A. elegans (leucosphyrus)	1	16
A. maculatus	2	6
A. majidi	0	7
A. pallidus	8,820*	242
A. philippinensis	172	6
A. splendidus	7	53
A. tessellatus	63	14
A. subpictus	451	49
A. turkhudi	2	0
A. vagus	408	55
A. varuna	4	12
Total	14,949	9,905

* *Only 38 adults in the high rainfall area and 6,947 in the low rainfall areas.*

larvae and adult collections. However, it should be noted that numbers of larvae collected depend not only upon habits but also on the number and variety of breeding places actually searched. It is normally difficult to correlate the numbers collected as larvae and the numbers collected as adults.

(6) North Kanara District, Karnataka State

North Kanara formerly of Bombay State, was surveyed by Jaswant Singh and Jacob (1944). Previously the district had been surveyed by Mhaskar (unpublished,) but he had failed to recognise the importance of *A. fluviatilis,* then called *A. listoni,* and had also concluded that *A. culicifacies* was the chief vector on epidemiological grounds. Both these conclusions have been found to be incorrect. Jaswant Singh and Jacob recorded 22 species of anophelines in the district, viz:

A. aitkenii
A. annularis
A. barbirostris
A. maculatus
A. nigerrimus
A. pallidus

A. culicifacies
A. culiciformis
A. fluviatilis
A. insulaeflorum
A. jamesii
A. jeyporiensis
A. karwari
*A. "leucosphyrus"**
A. philippinensis
A. stephensi
A. subpictus
A. tessellatus
A. theobaldi
A. turkhudi
A. vagus and
A. varuna

*The present author has seen both *balabacensis* and *elegans* from this district.

A. jeyporiensis was the most predominant species accounting for over a third of all the anopheline adults collected. Out of 30,616 mosquitoes of all species, they found only 1,010 *A. fluviatilis* and 1,282 *A. culicifacies*. On the basis of dissections they incriminated *A. fluviatilis* as the vector. Out of 897 dissections, 99 were found positive giving a total infection rate of 11 per cent (gut positives 6.2 per cent and gland positives 7.1 per cent).

In a concurrent and continuing study made by the workers of the Bombay Malaria Organization, 116 of the 1,634 *A. fluviatilis* dissected were found positive of which 45 showed only oocysts, 49 only sporozoites and 22 both oocyst and sporozoites giving an average infection rate of about 7 per cent. During some months and in certain localities natural infection rates as high as 40 per cent have been found (Viswanathan, 1950).

Among *A. culicifacies* of which 812 were dissected by Jaswant Singh and Jacob and 1,816 by the Bombay workers, none was found positive. No other species was positive. The Bombay workers collected 42,960 adults of all species of which 16,812 were *A. jeyporiensis* and 2,144 *A. fluviatilis* and 1,816 *A. culicifacies*. In addition to the species reported by Jaswant Singh and Jacob (*loc. cit.*) the Bombay workers found one *A. aconitus* but did not record *A. stephensi*.

7. Goa

The anopheline collections in Goa have been recorded by early workers such as James and Liston, DeMello and D'Sa, and Borkar and De Silva. They had found 18 species.

Subsequently Borkar *et al.* (1967) have listed the anophelines collected in Goa after the inception of the National Malaria Eradication Programme in the State showing additional species. They have given the distribution of the species by altitudes which is as follows:—

1 to 500 feet ... *A. annularis*,
A. barbirostris,
A. "hyrcanus",
A. jamesii,
A. splendidus,
A. subpictus,

A. tessellatus, and
A. vagus.

500 to 1000 feet ... *A. annularis*,
A. barbirostris,
A. culicifacies,
A. fluviatilis,
A. "hyrcanus",
A. jamesii,
A. jeyporiensis,
A. karwari,
A. pallidus,
A. subpictus,
A. tessellatus,
A. vagus and
A. theobaldi.

1000 to 2000 feet ... *A. annularis*,
A. barbirostris,
A. fluviatilis,
A. jamesii,
A. jeyporiensis,
A. karwari,
A. kochi,
A. majidi,
A. pallidus,
A. philippinensis,
A. stephensi,
A. subpictus,
A. tessellatus and
A. vagus.

The total absence of *A. maculatus* is noteworthy.

No dissections have been reported but on the similarity of epidemiology in the adjoining North Kanara and Belgaum District and Savantwadi (Ratnagiri District) of Maharashtra, *A. fluviatilis* is perhaps the major or sole vector. It would be interesting to record that Dr. C. Strickland had visited Savantwadi before World War II and had suggested that *A. maculatus* was the vector merely on the basis that this species was found in the area. It is now known that *A. maculatus* plays no significant part in malaria transmission in this zone.

8. Thane District (Maharashtra)

The workers of the Bombay Malaria Organization collected the following species in a survey conducted in 1943:—

Species	No. of adults collected
A. aconitus	*
A. annularis	18
A. barbirostris	12
A. culicifacies	405
A. fluviatilis	792
A. "hyrcanus" = nigerrimus	6
A. jamesii	81
A. jeyporiensis	189
A. maculatus	*
A. moghulensis	*
A. pallidus	12
A. philippinensis	*
A. splendidus	1
A. subpictus	2,538
A. tessellatus	40
A. turkhudi	*
A. vagus and	9
A. varuna	6

*Subsequently found in the District.

Dissections showed that one *A. culicifacies* out of 174 (0.6%), and 23 out of 574 (4.0%) *A. fluviatilis* were positive for plasmodial infections.

ZONE III — DECCAN

1. Mandya (Karnataka State)

Rao and Nassiruddin (1945) had listed the following anopheline mosquitoes in the Irwin Canal Area (Mandya, Mysore State, now Karnataka).

A. aconitus
A. annularis
A. culicifacies
A. fluviatilis
A. "hyrcanus" (= *nigerrimus?*)
A. jamesii
A. jeyporiensis
A. pallidus
A. stephensi
A. subpictus
A. tessellatus
A. turkhudi
A. vagus and
A. varuna

During the epidemic year (1932) 37 *A. culicifacies* out of 1,151 specimens were found naturally infected, the infection rate being 3.2 per cent.

A. fluviatilis was also considered of importance in the transmission of malaria.

2. Tungabhadra Project Area (Formerly Madras State, Now in Karnataka and Andhra Pradesh)

A malaria survey of Bellary and Kurnool Districts, which were to be mainly benefited by the Tungabhadra Irrigation Project was made by Bhaskar Rao, Ramanatha Rao and Sundararaman (1946) in the years 1942-1945.

The following 15 species of anophelines were collected either as adults or larvae.

A. aconitus
A. annularis
A. barbirostris
A. culicifacies
A. fluviatilis
A. nigerrimus
A. moghulensis
A. pallidus
A. stephensi
A. subpictus
A. tessellatus
A. theobaldi
A. turkhudi
A. vagus and
A. varuna

Nearly 15,000 dissections (between September 1943 and May 1944) of anophelines were made. Of 8,344 *A. culicifacies* dissected, 13 were found infected and of 900 *A. stephensi*, two showed infections. (0.15 and 0.22 per cent respectively).

3. Dharwar, Bijapur, Poona, Sholapur, Nira Canal Zone, Nasik, West Khandesh and East Khandesh

The Bombay Malaria Organization carried out surveys in several districts of the Deccan of the old Bombay State. The anophelines collected (Viswanathan, 1950) are shown in Table 15a.

It will be noticed that *A. culicifacies*, *A. fluviatilis*, and *A. stephensi* all take part in malaria transmission in the Deccan.

The *A. stephensi* prevalence in Deccan is of the var. *A. mysorensis* and it is most prevalent in the rural areas.

Lindberg (1935), when making a survey for the Barsi Light Railway in the Deccan of Maharashtra, had detected 18 species of anophelines all of which were found later by the Bombay workers. *A. culicifacies* was the most predominant species followed by *A. stephensi*, *A. annularis* and *A. subpictus*.

4. Greater Poona

Viswanathan (1950) has listed the following anophelines for Greater Poona City (now Pune City) from the survey carried out in the year 1944.

A. annularis
A. culicifacies
A. "hyrcanus"
A. jeyporiensis
A. splendidus
A. subpictus
A. theobaldi
A. vagus
A. barbirostris
A. fluviatilis
A. jamesii
A. pallidus
A. stephensi
A. theobaldi
A. turkhudi

Table 15a. Anopheles of the Deccan (Viswanathan, 1950)

	Karnataka		Maharashtra			
Years of Survey	Dharwar 1945-46	Bijapur 1945-46	Poona, Sholapur, Nira Canal Zone. 1947-49	Nasik 1947-49	West Khandesh 1948-49	East Khandesh 1948-49
Species:						
A. annularis	2,801	611	37	605	1,162	1,029
A. barbirostris	25	25	5	7	5	5
A. culicifacies	383	29,531	45,176	46,157	63,368	22,205
A. fluviatilis	396	6,315	55,095	3,557	3,274	300
A. hyrcanus	88	8	8	1	—	1
A. jamesii	108	146	—	740	—	—
A. jeyporiensis	4	19	1	2,617	1	22
A. karwari	10	—	—	—	—	—
A. maculatus	—	1	—	2	—	—
A. moghulensis	—	—	—	952	6	—
A. pallidus	494	64	6	105	12	44
A. philippinensis	14	—	—	—	—	—
A. splendidus	—	56	10	606	—	5
A. stephensi	405	8,373	57,279	1,824	5,673	1,565
A. subpictus	1,024	6,969	36,519	8,675	44,016	16,359
A. tessellatus	—	43	4	81	—	5
A. theobaldi	2	3	13	94	16	57
A. turkhudi	7	163	2,023	3,995	3,665	330
A. vagus	102	399	338	90	—	12
A. varuna	22	64	189	3	—	8
Total	5,885	52,790	196,773	70,111	121,198	42,947

The anophelines incriminated as vectors of human malaria in Deccan are shown in Table 15b.

Table 15b. Dissections in the Deccan

Species	Dharwar	Bijapur	Pune	Nasik	West Khandesh	East Khandesh
A. culicifacies	4/119*	9/3453	0/5154	5/10057	0/6056	7/2786
A. fluviatilis	0/168	1/980	5/6202	3/1475	0/232	0/25
A. stephensi	0/50	1/1272	1/4706	0/301	0/397	0/157

* Number positive/Number dissected.

Of 400 *A. culicifacies* dissected in May—June 1944, four were found infected (1.0 per cent). About 400 specimens of other four anophelines, *viz., A. fluviatilis, A. turkhudi, A. subpictus* and *A. stephensi* were dissected and no infection was found.

A. culicifacies was the main vector. This species was found breeding in all kinds of water, the exception being the deep impounded waters above the bund in the Mutha-Mula river.

The role of *A. stephensi* in malaria transmission requires further study.

Ramachandra Rao and Rajagopalan (1957) when making a survey of the mosquitoes in Poona District noted that the following anopheline species were actually found to bite man:

A. annularis
A. culicifacies
A. hyrcanus
A. karwari
A. moghulensis
A. tessellatus
A. barbirostris
A. fluviatilis
A. jamesii
A. maculatus
A. subpictus
A. theobaldi
A. vagus

Earlier Barber and Rice (1938), working in the city, had incriminated *A. culicifacies* as the vector.

5. Nizam Sagar Ayacut, Hyderabad State (now in Andhra Pradesh)

Abraham and Samuel (1944) surveyed the Nizam Sagar Ayacut. They detected 15 species of anophelines, viz:

A. aconitus
A. barbirostris
A. fluviatilis
A. nigerrimus
A. splendidus
A. subpictus
A. theobaldi
A. annularis
A. culicifacies
A. jamesi
A. pallidus
A. stephensi
A. tessellatus
A. turkhudi
A. vagus

They incriminated *A. fluviatilis* as the vector: one out of 117 dissected (0.86 per cent). They found no positives in 1,442 *A. culicifacies* and 1,829 *A. annularis* dissected.

6. Khandwa Tahsil—Nimar District of Madhya Pradesh

Subramaniam and Dixit (1948) carried out a survey in Khandwa Tahsil between July 1948 and January 1949, and collected the following species of anopheline mosquitoes:

A. annularis
A. fluviatilis
A. stephensi
A. turkhudi
A. culicifacies
A. splendidus
A. theobaldi
A. varuna

In all 8,943 specimens of the above species were dissected. Infections were found only in *A. culicifacies* and *A. fluviatilis*. Out of 7,337 *A. culicifacies* dissected, 10 were found with sporozoite infection (infection rate 0.21 per cent) while out of 1,324 *A. fluviatilis* dissected 17 showed sporozoite infection (infection rate 1.28 per cent). *A. fluviatilis* was mainly prevalent from July to September.

ZONE IV — GANGETIC VALLEY AND DELTA

1. Dhanbad Coal Mine Area

In a study of seasonal prevalence of anophelines in Dhanbad area (from July 1953 to May 1958), Sen *et al.* (1960) recorded the following 11 species of anophelines:

A. annularis	(6,671)
A. barbirostris	(4)
A. culicifacies	(4,374)
A. fluviatilis	(7,490)
A. "hyrcanus"	(50)
A. pallidus	(4,313)
A. splendidus	(534)
A. stephensi	(2)
A. subpictus	(9,095)
A. tessellatus	(1)
A. vagus	(167)
Total	33,054

(The figures in brackets are the numbers of adults collected)

A. annularis, A. culicifacies, A. fluviatilis, A. pallidus and *A. subpictus* formed over 95 per cent of the catches. *A. annularis* was found throughout the year but predominantly in late winter and early spring. *A. culicifacies* was most abundant during monsoon, from July to September, with a peak in August and again a small rise in February. *A. fluviatilis* was second only to *A. subpictus* in abundance. This species was found to be prevalent in high densities from November to December. *A. subpictus* was the most dominant species and found throughout the year with a definite seasonal abundance during the monsoon months. *A. annularis* was found only during late winter. *A. pallidus* was found throughout the year but the peak densities were during early winter (November).

In the above study, the incrimination of malaria vectors in the area was not undertaken. However, Rao (1944) as quoted by Sen *et al.* (*loc. cit.*) has recorded the

following species as malaria vectors in the Jharia coal mining settlement, south eastern part of Bihar State.

A. annularis—Sole vector in south eastern corner of Jharia mining settlement with a gut infection rate of 0.5 per cent and gland infection rate of 0.1 per cent.

A. culicifacies—In dissections of 1,509 specimens over a period of 5 years, the infection rates were 0.6 per cent for oocysts and 0.1 for sporozoites. Rao considered that *A. culicifacies* was relatively unimportant.

A. fluviatilis—The species was found to be a vector of primary importance.

A. pallidus—A gut infection of 0.8 per cent and a gland infection of 0.1 per cent were recorded, but the species was considered relatively unimportant.

A. stephensi—The species was incriminated as a vector of secondary importance.

2. Malaria Vectors in Bengal

Iyengar (1940), in the dissections of mosquitoes made in Bengal between 1936 to 1939, found the following species infected:—

Species	Infection rate
A. philippinensis	6.3 per cent
A. minimus	18.6 per cent
A. annularis	0.04 per cent
A. varuna	0.2 per cent
A. sundaicus	15.8 per cent

The important malaria vectors in Bengal were: *A. philippinensis* in the plains, *A.minimus* in the foothills and *A. sundaicus* in the esturine regions.

Further Iyengar (1944) dissected 9,705 anophelines belonging to 12 species in different parts of deltaic Bengal. (Table 16)

Table 16. Dissections in Deltaic Bengal. (Iyengar, 1944)

Species	No. dissected	No. positive
A. aconitus	1,339	Nil
A. annularis	2,600	Nil
A. barbirostris	49	Nil
A. culicifacies	9	Nil
A. nigerrimus	480	Nil
A. pallidus	71	Nil
A. philippinensis	865	64 (7.4 per cent)
A. ramsayi	742	1
A. subpictus	570	Nil
A. tessellatus	11	Nil
A. vagus	2,607	Nil
A. varuna	536	Nil

The past role of *A. sundaicus* as a vector in Bengal is very well known. The papers by Roy, Iyengar, and Sen provide numerous references.

It was an important vector in Sunderbans and also in the Salt lake area near Calcutta. A study of the Salt lake area was made by the Calcutta School of Tropical Medicine under a scheme sanctioned by the Indian Research Fund Association. The area has now become a part of Calcutta City.

3. Bengal-Orissa Border

Ganguli (1947) has reported the following species of anophelines in the Bengal-Orissa border.

A. aconitus
A. barbirostris
A. nigerrimus
A. philippinensis
A. subpictus
A. annularis
A. culicifacies
A. pallidus
A. ramsayi
A. vagus
A. varuna

A. philippinensis was the vector in the area. Out of 294 specimens of the species dissected, 12 were found infected (4.1 per cent). No infection was found in *A. annularis.*

According to Iyengar (1944), *A. philippinensis* in deltaic areas in Bengal was supposed to have a limited seasonal activity and Ganguli corroborated it.

4. Punjab, Delhi, Haryana and Uttar Pradesh

The States of Punjab, Haryana, Uttar Pradesh and the Union Territory of Delhi have received considerable attention. However, comprehensive surveys for any particular locality have been scanty. Delhi City has been the subject of many papers. The anopheline fauna of Delhi are the same as in the adjoining areas of Punjab and Haryana. The mountain and sub-mountain areas of these states have been the subject of detailed surveys and they are referred to under the Himalayan Zone.

An early survey by Macdonald and Majid (1931) and another by Hicks and Majid (1937) in Karnal District, may be mentioned. Macdonald and Majid found 11 species of which *A. annularis* was the most abundant followed by *A. culicifacies* and *A. subpictus.* Dissections revealed 9.8 per cent gut infections in *A. culicifacies,* 8.3 per cent in *A. fluviatilis,* 3.8 per cent in *A. splendidus* and 0.5 per cent in *A. annularis.* Glands were not dissected.

Hicks and Majid followed up the studies and found 14 species as follows:

A. subpictus	35,987	*A. hyrcanus*	42
A. annularis	21,772	*A. barbirostris*	5
A. culicifacies	8,344	*A. turkhudi*	3
A. pallidus	1,407	*A. maculatus*	1
A. fluviatilis	873	*A. jamesi*	1
A. stephensi	483	*A. gigas*	(One larva)
A. pulcherrimus	297		
A. splendidus	268		

The species composition in the entire upper Gangetic valley (plains) is almost similar to the above. *A. culicifacies* is the principal vector with *A. stephensi* playing some role in urban areas.

ZONE V — EAST CENTRAL INDIA

1. Jeypore Hills

The Jeypore hills form a part of the Eastern Ghats range. Senior White (1937) carried out dissections of anophelines in 1935-1936 in this hill region and collected the following 23 species.

A. aconitus
A. annularis
A. culicifacies
A. nigerrimus
A. jeyporiensis
A. maculatus
A. minimus
A. pallidus
A. splendidus
A. subpictus
A. theobaldi
A. aitkenii
A. barbirostris
A. fluviatilis
A. jamesii
A. karwari
A. majidi
A. moghulensis
A. philippinensis
A. stephensi
A. tessellatus
A. vagus
A. varuna

The funestus groups consisting of *A. fluviatilis, A. varuna* and *A. minimus* had the highest incidence from September to February. The group was mainly attracted to human dwellings and overflowed into cattle sheds only in the peak months.

A. culicifacies rested predominantly in cattle sheds. The species was most prevalent in the spring and in the rains.

A. jeyporiensis was found resting mostly in cattle sheds. It had peak prevalence in November to March. During August and September (heavy rainfall months) the species disappeared altogether.

A total of 6,944 specimens consisting of the following species were dissected:

A. culicifacies (4,744)
A. varuna (317)
A. aconitus (107)
A. splendidus (38)
A. theobaldi (19)
A. pallidus (68)
A. fluviatilis (1,111)
A. minimus (201)
A. jeyporiensis (318)
A. maculatus (13)
A. jamesii (2)
A. annularis (6)

Infections were found in *A.culicifacies* (oocyst rate 0.60%), *A. fluviatilis* (oocyst

rate 7.2%, sporozoite rate 3.5%), *A. varuna* (oocyst rate 8.4%, sporozoite rate 4.7%), *A. minimus* (oocyst rate 8.5%, sporozoite rate 4.0%), and *A. jeyporiensis* (oocyst rate 1.3%).

The main vectors were the "*funestus*" group consisting of *A. fluviatilis, A. varuna* and *A. minimus.*

2. Singhbhum District

In Singhbhum District also, *A. fluviatilis, A. varuna* and *A. minimus* were vectors (Senior White and Das, 1938). None of the 1,611 *A. culicifacies* dissected was sporozoite positive. 1.8 per cent of *A. fluviatilis* (1,031), 1.1. per cent of *A. varuna* (189) and 3.9 per cent of *A. minimus* (334) were sporozoite positive.

Numbers collected in houses and cattle sheds during a 16 month period are very interesting:

	Houses	Cattlesheds
A. culicifacies	257	508
A. fluviatilis	204	5
A. varuna	2	1
A. minimus	180	2

The preponderance of *A. fluviatilis* and *A. minimus* in human dwellings is significant.

Senior White (1938) extending his studies on the malaria transmission in the Jeypore hills came to the conclusion that in that area *A. culicifacies* played no part, but *A. fluviatilis, A. varuna* and *A. minimus* were the vectors. Out of the 2,446 *A. culicifacies* dissected none was positive for sporozoites though two had oocysts. On the other hand *A. fluviatilis* had an oocyst rate of 6.8 per cent and sporozoite rate of 3.0 per cent in 760 dissected. *A. varuna* had an oocyst rate of 8.9 per cent and sporozoite rate of 4.0 per cent out of 225 dissected, and *A. minimus* 5.1 and 4.1 per cent respectively out of 7,195 dissected.

3. Hazaribagh Ranges Including Ranchi Plateau

Senior White (1943) during his investigations on malaria transmission in the Hazaribagh ranges recorded the following anopheline fauna which comprised 20 species.

A. aconitus
A. barbirostris
A. fluviatilis
A. jamesii
A. karwari
A. moghulensis
A. ramsayi
A. stephensi mysorensis
A. annularis
A. culicifacies
A. nigerrimus
A. jeyporiensis
A. maculatus
A. pallidus
A. splendidus
A. subpictus

A. theobaldi *A. tessellatus*
A. vagus *A. varuna*

The main vector is *A. fluviatilis* which transmits malaria from September to March. *A. culicifacies* is also responsible for transmission of the disease to some extent in the months of July and August/September.

ZONE VI — EASTERN REGION: ASSAM AND NEIGHBOURING STATES

1. Assam

Anderson and Viswanathan (1941) reported results of dissections prior to 1941 in Assam numbering 70,049 of several species of which 774 specimens showed infections (409 gland and 425 gut infections). Six species were found infected of which *A. minimus* was the most predominant (Table 17).

Reference was also made to infections in *A. balabacensis* in Digboi reported by Clark and Choudhary (1941).

In a further series of dissections totalling 18,599 anophelines (Viswanathan *et al.* 1941), *A. minimus* was found to be the most important vector. The figures for 1941 are also shown in Table 17.

Table 17. Dissections in Assam
ANDERSON AND VISWANATHAN (1941)

	Dissected	Positive		Total infections	Total infection rate
		Gut	Gland		
Prior to 1941					
A. aconitus ..	1,145	1	—	1	0.1
A. annularis ..	16,760	3	6	9	0.1
A. culicifacies ..	1,232	2	5	6	0.5
A. maculatus var. *willmorei* ..	8483	24	5	29	0.3
A. minimus ..	14,092	383	381	725	5.2
A. philippinensis ..	4,239	2	2	4	0.1
1941 results					
A. minimus ..	5,120	72	83	154	3.0
A. maculatus ..	1,573	1	13	14	0.9
A. annularis ..	7,481	7	4	11	0.15
A. aconitus ..	254	—	1	1	0.4

Discussing these results and comparing those with the data for earlier years, they regarded that *A. minimus* was the most important vector in Assam with *A. maculatus*, a minor vector in Shillong and *A. annularis* to be vector in certain parts of Golpara District.

A. culicifacies was not reckoned to be a vector of any importance in Assam though five gland positives were found in the earlier studies.

Similarly, though *A. philippinensis* had been found naturally infected previously, it was not regarded as of much importance. *A. minimus* occurred throughout the year with high densities in the months of June to November but natural infections were found in all months except February, with a peak in March.

These findings regarding *A. philippinensis* and *A. minimus* are very important in view of the changes which have taken place regarding their prevalence and behaviour in recent years.

2. Manipur

Mortimer (1946) in his survey of anopheline fauna of Manipur area during the period November 1943 to March 1944, found the following 16 species.

A. ahomi
A. aitkenii var. *bengalensis*
A. gigas
A. jeyporiensis var. *candidensis*
A. maculatus
A. pallidus
A. sinensis
A. aitkenii
A. annularis
A. gigas var. *bailey*
A. lindesayi
A. nigerrimus
A. philippinensis
A. vagus

The collections were made at altitudes ranging from 2,500 to 4,250 feet (750 to 1,380 metres). There is no record of dissections.

The total absence of *A. balabacensis* and *A. minimus* is noteworthy.

3. Tripura State

Misra and Dhar (1955) carried out a survey in Tripura State from January to May 1954, and collected the following adult anophelines.

A. aconitus
A. barbirostris
A. fluviatilis
A. jeyporiensis
A. subpictus
A. annularis
A. culicifacies
A. hyrcanus
A. minimus
A. vagus

In all, 1,508 anophelines were collected. *A. vagus* was the most predominant species forming more than 80 per cent of the total collections. *A. minimus* formed 13 per cent.

Two out of 99 *A. minimus* dissected were found infected with sporozoites.

4. Meghalaya

Rajagopal (1976) in his studies on the persistent malaria transmission in Burni-

hat, Meghalaya, in 1968 (June-September) found 14 species of anophelines, all during night collections while biting man or cattle, both outdoors and indoors.

A. aconitus	..	32	
A. annularis	..	122	
A. balabacensis	..	2	
A. barbirostris	..	7	
A. hyrcanus	..	454	(Whether they were entirely *nigerrimus* or included related forms is not known).
A. jamesii	..	3	
A. jeyporiensis	..	4	(Any *candidiensis*?)
A. karwari	..	71	
A. kochi	..	180	
A. maculatus	..	9	
A. minimus	..	1	
A. philippinensis	..	1,622	
A. tessellatus	..	2	
A. vagus	..	88	
	..		
Total	..	2,597	
	..		

A. philippinensis was the most abundant species, but it was never found resting during day time inside houses and cattle sheds. Only one adult of *A. minimus* was found. It had certainly undergone a great reduction in its prevalence in this area. No adults of any species were collected inside houses which had been sprayed with DDT.

Out of 174 *A. philippinensis* dissected one specimen showed sporozoite infection (0.48 per cent).

The extreme rarity of *A. balabacensis* and *A. minimus* is noteworthy.

ZONE VII—COASTAL ORISSA, ANDHRA PRADESH AND TAMIL NADU

1. Chilka Lake Area (a)

Chilka lake in Orissa has been the venue of many investigations because of the role of *A. sundaicus* in malaria transmission. The lake is a large bay cut off from the Bay of Bengal by drift of sand along the coast and is now separated from the actual sea by a sand ridge. The total area of the lake is about 350 sq. miles (836 sq. km.). The intense malaria prevailing in the vicinity of this lake had been investigated (in 1912) when spleen rates of over 80 per cent and parasite rates of over 50 per cent had been found but the vector was not definitely known. Among others who had investigated this area was Sarathi (1932) under the auspices of the IRFA. But he did not detect *A. sundaicus* though he specially looked for it. Annandale and Kemp (1915) (quoted from Venkat Rao, 1961) made a zoological survey of the area and were able to find *A. rossi* which is now identified as *A. subpictus*.

A. sundaicus was first found in the area at the village of Konaka on 31 March 1937 by a team working under Senior White. They (Senior White and Adhikari, 1939) found 20 species of anophelines:

A. subpictus	*A. karwari*
A. vagus	*A. splendidus*
A. sundaicus	*A. ramsayi*
A. maculatus	*A. jamesii*
A. fluviatilis	*A. tessellatus*
A. varuna	*A annularis*
A. aconitus	*A. philippinensis*
A. jeyporiensis	*A. pallidus*
A. maculatus	*A. hyrcanus*
A. theobaldi	*A. barbirostris*

In a large series of dissections made in five localities, they found 10 gut positives and 5 gland positives in 659 *A. sundaicus*. They also found one infected *A. aconitus* out of 47 dissected. They definitely incriminated *A. sundaicus* as a vector of malaria in the area. Senior White had already reported the occurrence of *A. sundaicus* in this area and attributed it to be a new invasion from lower Bengal.

Chilka Lake Area (b)

One of the most careful and intense surveys of anophelines and malaria was that carried out by Covell and Pritam Singh (1942) in the coastal belt of Orissa, particularly around Chilka lake, for 3 years between 1939 and 1942. Chilka Lake and its relation to malaria was the main objective of the survey. 17 species of anopheline were encountered *viz.*,

A. aconitus	*A. pallidus*
A. annularis	*A. ramsayi*
A. barbirostris	*A. stephensi*
A. culicifacies	*A. subpictus*
A. fluviatilis	*A. sundaicus*
A. nigerrimus	*A. tessellatus*
A. jamesii	*A. vagus*
A. karwari	*A. varuna*
A. maculatus	

A. sundaicus was the most important species and was also the only species in which plasmodium infections were found. 12 gut and 57 gland infections were found out of 10,714 dissected. This species was mainly breeding in brackish waters. It was also found in some fresh water breeding places a little away from the coast.

2. Ennore-Nellore Region (North of Madras City)

Russell and Jacob (1939a) reported their findings in the Ennore-Nellore region. They dissected 12 species *viz.*,

A. annularis	*A. pallidus*

A. barbirostris
A. culicifacies
A. jamesii
A. karwari
A. nigerrimus
A. stephensi
A. subpictus
A. tessellatus
A. vagus
A. varuna

A. culicifacies was the vector, 6 positives (1 gland positive and 5 gut positives) being found among 984 dissections (0.61%). The breeding of the species in pits in casuarina plantations was perhaps the main cause for the malaria prevalence.

The paper gives an excellent review of the previous literature on anophelines of the region.

3. Pattukkottai Taluk (Tamil Nadu)

Russell and Ramachandra Rao (1941a) in their extensive studies on the anophelines of Pattukkottai Taluk found 12 species. The seasonal prevalences of all the 12 species are summarized below:

Species	*Greatest prevalence*	*Comments*
A. aconitus	February to March.	Absent in months May to December.
A. annularis	March to July, peak in April and May.	But occurs throughout the year.
A. barbirostris	November to March, peak in January.	Very rare in other months.
A. culicifacies	July to October, peak in August.	Occurs throughout the year; can thrive from February to June, provided there are suitable breeding places. The only malaria vector.
A. nigerrimus	November to January, peak in December to January.	Very scarce in months of February to June.
A. jamesii	January to May.	Occurs throughout the year.
A. pallidus	September and October, peak in September.	Scarce in months February to June, but can thrive if suitable habitats available.
A. stephensi	No seasonal prevalences noticed.	
A. subpictus	July to November, peak in August.	Occurs throughout the year and thrives from February to June if there are breeding places.
A. tessellatus	December to February, peak in December.	Scarce in other months.
A. vagus	August to November, peak in October.	Occurs throughout the year and can thrive from February

		to June, if breeding places available.
A. varuna	No seasonal variations noted.	Occurs throughout the year.

The Pattukkottai area was re-surveyed in 1978, after a lapse of 38 years by Rajagopalan *et al.* (1979) and Chandrahas *et al.* (1979). They found a great reduction in the prevalence of *A. culicifacies* even during the irrigation season. *A. subpictus* had, however, increased substantially in prevalence. While formerly it was of the same abundance as *A. culicifacies,* it was now 18 times more. There is, however, no prevalence of malaria.

4. Orissa Coastal Plains

Orissa coastal plains extend from the northern boundary of Orissa to Puri. Senior White *et al.* (1943) working on malaria transmission in the area during 1935 to 1941, found the following 18 species of anophelines:

A. aconitus
A. barbirostris
A. fluviatilis
A. jamesii
A. karwari
A. philippinensis
A. subpictus
A. sundaicus
A. vagus
A. annularis
A. culicifacies
A. hyrcanus
A. jeyporiensis
A. pallidus
A. ramsayi
A. splendidus
A. tessellatus
A. varuna

Only one specimen of *A. fluviatilis* was collected and it was believed to have been transported passively by train. *A. annularis,* the most dominant species, in the area, formed 70 per cent of all the collections. *A. aconitus* was most prevalent from November to March. However, the greatest density was from January to March. *A. sundaicus* was very rarely found, other than around Puri.

12,987 dissections of the thirteen species caught were made. Only two species, *A. aconitus* and *A. annularis* were found with sporozoites. The dissections showed the infection rates given in Table 18.

Table 18. Dissections in Orissa plains by Senior White

Species	No. dissected	Positives		Infection rate, per cent
		Gut	Gland	
A. culicifacies	735	1	—	0.1 (gut)
A. aconitus	951	3	3	0.3 (gut)
				0.2 (gland)
A. ramsayi	556	1	—	0.2 (gut)
A. annularis	9,183	14	7	0.15 (gut)
				0.08 (gland)
A. pallidus	1,409	1	—	0.7 (gut)

The principal vector was *A. annularis.* The transmission season by this species was from September to November/December. *A. aconitus* was also a vector in certain areas. Transmission season was from September to December.

5. Visakhapatnam and Neighbourhood

Investigations into malaria transmission around Visakhapatnam were carried out by senior White and Venkat Rao (1943).

The local fauna consisted of 22 species of anophelines.

A. aconitus	*A. annularis*
A. barbirostris	*A. culicifacies*
A. fluviatilis	*A. hyrcanus*
A. jamesii	*A. jeyporiensis*
A. karwari	*A. maculatus*
A. minimus	*A. moghulensis*
A. pallidus	*A. philippinensis*
A. ramsayi	*A. splendidus*
A. stephensi	*A. subpictus*
A. tessellatus	*A. theobaldi*
A. vagus	*A. varuna*

The main malaria vector was *A. stephensi mysorensis* with a sporozoite rate of 1.75 per cent. The species was found infected in August, October, November, January and February.

A. culicifacies was considered a vector of minor importance as only one was found with sporozoite infection (sporozoite rate of 0.15 per cent). Similarly, *A. varuna* had a very low infection rate (sporozoite rate of 0.17, per cent) and was also considered of minor importance in malaria transmission.

ZONE VIII—HIMALAYAN ZONE

1. Kashmir (a)

Jacob (1950) surveyed some parts of Jammu and Kashmir State and found the following anopheline species.

Ladakh:	No specimen.
Gulmarg:	*A. gigas* and *A. moghulensis.*
Central plateau:	*A. annularis, A. splendidus, A. stephensi, A. subpictus* and *A. fluviatilis.*
Western portions, *i.e.*, in the valley of Jhelum and Kishan Ganga:	All the above and *A. lindesayi, A. dthali, A. turkhudi, A. culicifacies* and *A. maculatus.*
Foothills south of Pirpinjal range:	Similar to western zone.

A. culicifacies common in the Jammu region and was noted as a vector but it was not encountered at locations above 4,000 feet (1,300 metres) MSL. *A. fluviatilis* was regarded as a vector at the high altitudes.

Kashmir (b)

In Kashmir, Nair (1973) found the following species:

A. annularis
A. culicifacies
A. fluviatilis
A. "hyrcanus" (? nigerrimus)
A. "leucosphyrus" (? A. balabacensis)
A. maculatus
A. splendidus

A. maculatus was most prevalent followed by *A. fluviatilis* and *A. annularis*. One *A. fluviatilis* out of 56 dissected showed sporozoites, the location being between 1,676 and 1,829 metres above sea level. Previously, Jacob (1950) had found malaria transmission at a height of 1,829-1,981 metres. Nair found unstable but low malaria prevalence upto 2,134 metres.

The densities of *A. fluviatilis* (0.33 to 5.3 per man hour) and *A. maculatus* (0.33 to 18.7 per man hour) were quite high.

The report of *A. leucosphyrus* (*A. balabacensis* ?) is very interesting. It had not been reported in this State so far.

2. U.P. Terai

Issaris *et al.* (1953) during a malaria survey in U.P. Terai found the following species:

A. aconitus
A. barbirostris
A. fluviatilis
A. karwari
A. minimus
A. pallidus
A. stephensi
A. vagus
A. umbrosus
A. annularis
A. culicifacies
A. gigas (var. *simlensis*?)
A. maculatus (var. *willmorei*)
A. nigerrimus
A. splendidus
A. subpictus
A. varuna

(NOTE: *A. umbrosus* has not been collected by others west of Assam).

A. minimus which was earlier regarded as the main malaria vector was very rare. *A. culicifacies* and *A. fluviatilis* constituted the bulk of the species collected. 7,806 *A. fluviatilis* were dissected and seven were found positive. The species was present throughout the year with a peak in June to October. *A. culicifacies* was also prevalent from June to October. Out of 5,742 dissected, five gland infections were found. *A. fluviatilis* had a higher anthropophilic index (41.2 per cent; 63 per cent in houses, 44.7 per cent in outdoor shelters and 28.4 per cent in cattle sheds).

3. Himalayas

Ramachandra Rao *et al.* (1973) reported results of an extensive survey of the entire Himalayan region over a period of three years (Table 19). Later Bhat (1975 a, 1975b) described in detail the locations and altitudes where the species were found.

Table 19. Anophelines in Himalayas, Ramachandra Rao *et al.* (1973)

	Jammu & Kashmir	Himachal Pradesh	Uttar Pradesh	West Bengal & Sikkim
A. aitkenii ..			*	
A. annularis ..	*	*	*	
A. culicifacies ..	*	*	*	
A. culiciformis ..			*	
A. fluviatilis ..	*	*	*	
A. gigas bailey ..				*
A. gigas simlensis ..	*	*	*	
A. jeyporiensis ..			*	
A. lindesayi ..	*	*	*	*
A. maculatus willmorei ..	*	*	*	*
A. nigerrimus ..			*	
A. pulcherrimus ..			*	
A. splendidus ..	*		*	
A. stephensi ..			*	
A. subpictus ..	*		*	
A. theobaldi ..			*	*
A. turkhudi ..	*			
A. vagus ..			*	

(* = present)

The highest location where any species of *Anopheles* was found was at Kedarnath (3,530 metres; *A. gigas simlensis*).

The most widely distributed species were *A. maculatus* var. *willmorei, A. gigas* var. *simlensis* and *A. lindesayi.*

No dissections were made to determine the vectors. The highest elevation where *A. fluviatilis* was found was 1,070 metres and *A. culicifacies* 1,300 metres.

Not a single specimen of either *A. minimus* or *A. balabacensis* was found.

4. U.P. Himalayas

Wattal *et al.* (1967a) in their survey of Nainital District comprising hilly terrain, Bhabar and Terai, collected four species of anophelines.

Hilly terrain altitude ranging from 542 metres to 1930 metres:

A. culicifacies —	altitude	1,524 metres
A. stephensi —	"	1,525 metres
A. subpictus —	"	542 to 1,220 metres

Bhabar area:

A. culicifacies —	altitude	259 to 266 metres
A. fluviatilis —	"	226 metres
A. stephensi —	"	226 metres
A. subpictus —	"	226 to 488 metres

Terai:

A. culicifacies —	altitude	210 metres
A. fluviatilis —	"	210 metres
A. stephensi —	"	208 metres
A. subpictus —	"	208 metres

5. West Bengal and Sikkim—Himalayas

Varma and Mahadevan (1970), during a survey of the eastern Himalayan foothills in a belt of about 200 kms. in the Darjeeling, Kurseeong, Kalimpong, and Bhutan foothills, during July to December 1966, found the following species:

A. aconitus
A. annandelei
A. culiciformis
A. jamesii
A. kochi
A. lindesayi
A. majidi
A. philippinensis
A. sintoni
A. vagus
A. aitkenii
A. barbirostris
A. gigas (var. ?)
A. karwari
A. leucosphyrus
A. maculatus var. *willmorei*
A. nigerrimus
A. splendidus
A. tessellatus

NOTE: *A. kochi* was the most prevalent species of *Anopheles*. The absence of *A. minimus* may be noted.

(See under 'Nepal' in the Chapter dealing with anophelines of neighbouring countries for further information on the Himalayan region.)

6. Arunachal Pradesh

Misra (1956) made a brief survey of this region and collected the following species:

A. aconitus
A. maculatus
A. pallidus
A. annularis
A. minimus
A. vagus

The survey was done in an unfavourable season, *viz.*, winter months. It was noted that *A. minimus* constituted 9.7 per cent of the total collections. One out of 7 dissected was found gland positive. Misra did not collect *A. balabacensis*, but he thought it possibly occurred.

7. Tirap region

Sen *et al.* (1973) carried out a survey in Tirap region from February to November, 1969 and collected following species:

A. aconitus
A. balabacensis
A. jeyporiensis (var. ?)
A. kochi
A. pallidus
A. splendidus
A. vagus
A. annularis
A. barbirostris
A. nigerrimus
A. maculatus (var. ?)
A. philippinensis
A. subpictus
A. varuna

These were mostly collected on human baits, and *A. balabacensis* formed 60 per cent of 3,407 anophelines collected. It may be noted that no *A. minimus* adults were collected. There were 3 gland positives, in 1,811 *A. balabacensis*. Out of a total of 3,376 of other anophelines (total 11 species) dissected, no mosquito was found with a gut or gland infection.

ZONE IX—OUTLYING ISLANDS

1. The Andaman and Nicobar Islands

(a) The Andaman Islands had been surveyed as far back as 1912 by Christophers and later by Covell in 1927a. The importance of *A. sundaicus* as a vector had been recognized.

In recent years more extended surveys have been done in both the Andaman and Nicobar Islands.

(b) In a mosquito survey in the Andaman Island in 1965, Krishnan and Herlenkar (1967) collected the following anophelines:

South Andaman (Mile Tilak area)	*A. aconitus, A. balabacensis, A. barbirostris, A. kochi, A. maculatus, A. philippinensis, A. sundaicus, A. tessellatus* and *A. vagus.*
Middle Andaman (Rangat)	*A. aconitus, A. balabacensis, A. barbirostris, A. kochi, A. maculatus, A. philippinensis, A. sundaicus* and *A. vagus.*
North Andaman (Rest Camp and Webi)	*A. kochi, A. maculatus, A. philippinensis, A. sundaicus* and *A. vagus.*

Nine species of anophelines were thus collected in the Islands as a whole. In a further survey in Car Nicobar Island, Krishnan and Bhatnagar (1968) found only two species, *viz., A. sundaicus* and *A. barbirostris.*

More mosquitoes were collected during the night than during daytime catches. *A. balabacensis, A. barbirostris, A. maculatus, A. philippinensis* and *A. tessellatus* were collected at night only. *A. sundaicus, A. vagus, A. aconitus* and *A. kochi* were collected both during night and day.

Anophelines mosquitoes were comparatively less in number in indoor shelters

during the day; only 8 per cent of the total collection of all mosquitoes was found resting there.

In all 1,042 anophelines were dissected for sporozoites and oocysts, all were found negative. But *A. sundaicus* and *A. balabacensis* are regarded as vectors.

Subsequently, workers of N.I.C.D. carried out a mosquito survey in the Andaman and Nicobar Islands (N.I.C.D. Annual Report, 1970 & 1975). In addition to the species of anophelines collected by Krishnan and Herlenkar (*loc. cit.*) and Krishnan and Bhatnagar (1968) the N.I.C.D. workers, collected *Anopheles barbumbrosus* and probably, *A. hodgkini*. The identification of the last species requires further examination of eggs and immature stages (The final report has not yet been published).

Very recently (Kalra, 1980) the probability of a species of monkey malaria being transmitted to man has been postulated (see under *A. sundaicus*).

2. Lakshadweep

(a) Nair (1961) records the occurrence of five species of anophelines in the Lakshadweep including those previously found by the workers of the Madras Public Health Department. No anopheline had been found in Minicoy. Table 20 shows the species found in the different Islands till 1961.

Table 20. Anophelines of Lakshadweep (1961)

	Minicoy	Kiltan	Chetlet	Amin	Kadamath	Agathi	Kavarathi	Androt	Kalpani
A. varuna		*	*		*	*	*	*	
A. subpictus		*		*			*	*	*
A. vagus		*		*			*	*	*
A. hyrcanus									*
A. barbirostris									*

* Present.

(b) Roy *et al.*(1974) recorded the results of malaria survey carried out in the Minicoy Island in November 1973. They found malaria prevalent and 58 out of 2,881 blood smears collected were positive. Entomological surveys showed no adult anophelines inside houses but six specimens of *A. tessellatus* were collected during night resting under the eaves of houses. Larvae and pupae of the same species were found breeding in wells and soak pits. *A. tessellatus* was suspected to be the vector on the analogy of the Maldives. Possibly the vector was passively transported from the Maldives.

(c) Roy *et al.* (1978) have reported that in the Lakshadweep Island, malaria which had disappeared had re-appeared in 1972. In Minicoy Island, *A. tessellatus* was regarded as the vector, the control of which again brought down the incidence of the disease.

However, Bitra Island was affected in 1976. *A. subpictus* was found breeding in both fresh and brackish waters (45.5 per cent of 123 persons were positive for *P.*

vivax). *A. subpictus* was suspected to be the vector on the analogy of the information in the Maldive Islands, (WHO unpublished information made available to Dr. Roy). *A. tessellatus* had disappeared in Maldives.

In Chetlet Island, *A. varuna* and *A. subpictus* had been previously found and also in other islands. They were both suspected to be vectors.

Therefore, the anophelines species so far found in Lakshadweep are: *A. varuna, A. subpictus, A. vagus, A. 'hyrcanus', A. barbirostris* and *A. tessellatus. A. varuna, A. subpictus* and *A. tessellatus* have been suspected to be vectors on epidemiological grounds.

12

The Anophelines of Neighbouring Countries

In the following pages an attempt has been made to provide complete lists of the anophelines of neighbouring countries both to the west and east of India. As far as possible reliance has been placed on the more recent survey reports and on world and regional catalogues. Brief information on the malaria vectors is also given for each country.

Some Species of Anophelines of Neighbouring Countries of Interest to Indian Workers

A. superpictus Grassi, 1899

A member of the subgenus *Cellia*, series *Neocellia*, it has wide distribution in whole of the West Asia, Mediterranean region, South Western USSR, Pakistan and Afghanistan (both North and South). It is a species which has been well studied. Adults are found abundantly in houses and stables and the species is mainly zoophilic but bites man readily. In winter the species may hibernate with development of fat bodies. It is regarded as a fairly strong flier.

This species breeds in Pakistan and Afghanistan in hill streams and irrigation water. Flowing water seems to be a necessity. Though the larvae are found in grassy margins of channels, an ideal breeding place is the bed of a river covered with rounded pebbles. Superficially the river appears to be dry, but when the pebbles are removed, larvae are found in the water below.

In Pakistan and Afghanistan, the larvae live in close association with *A. culicifacies* and *A. stephensi*. The species has been found at elevations of over 7000 feet (2100 metres) in Pakistan, but the present author has collected this species in valleys (200-600 metres elevation) in Afghanistan, both North and South.

There is a possibility that the species occurs in parts of Kashmir bordering NWFP of Pakistan and Afghanistan. It has been found in Kalat at an altitude of about 6,000 ft. (1,900 metres).

A. superpictus is well known as a malaria vector wherever it occurs. Infected specimens have been found in Greece, Macedonia, Bulgaria, Cyprus, Israel, Iraq, Pakistan and Afghanistan.The present author first detected its role in Afghanistan (Ramachandra Rao, 1951).

A. sergentii Theobald, 1907

A member of the series *Myzomyia* it is closely related to *A.culicifacies*. It has a

wide distribution in the entire Mediterranean (North Africa) and West Asian countries, and Pakistan. The adult is a house frequenting mosquito and has been found with human blood. It is sometimes found resting in underground aqueducts (Kerezes). It breeds in small pools and springs often under stones like *A. superpictus*. Though found breeding sometimes in rice fields and stagnant waters, it prefers flowing water. In Pakistan, it has been found breeding in irrigation channels and small pools in the river beds. It has occasionally been found infected with human plasmodia and has been regarded as a cause of some epidemics in Egypt and Palestine.

A. dthali Patton, 1905

A member of the series *Myzomyia*, it is related to *A. sergentii* and *A. culicifacies*. It is distributed widely in West Africa, North Africa, West Asia upto and including Pakistan. It has been recorded in Kashmir. It has not been recorded from Afghanistan yet but probably occurs there. It has been found resting outdoors during daytime and to feed on man. In Pakistan, it has been found breeding in springs and wells as also in pools in the river beds. Larvae have also been found in waters of underground aqueducts, tanks and wells and also in deep pools of water with vegetation. It is suspected to be a minor vector on epidemiological grounds, but supporting dissection records are lacking.

A. leucosphyrus Donitz, 1901

A member of the series *Neomyzomyia*, it is closely related to *A. balabacensis* and *A. elegans*. Formerly both these species were identified in India under the name *A. leucosphyrus*, but after Colless's work of 1956, they have now been separated. It has very similar habits to the two species and according to Knight and Stone (1977) it occurs in Sumatra, Kalimantan and Malaysia. However, Stone and Delphinado (1973) do not include it in their check list of oriental mosquitoes. There is doubt about its separate identity. Regarding *A. dirus*, see under *A. balabacensis*.

A. gigas var. refutans Alcock, 1913

It is a variety which occurs only in Sri Lanka. It has habits very similar to *A. gigas* and its varieties of India.

A. campestris Reid, 1962

A member of the *barbirostris* group, it is prevalent in Malaysia and Thailand. The adults are rather difficult to distinguish from those of *A. barbirostris*. Reid (1962 & 68) discussed the possibilities of the species occurring in India, Burma and 'Indochina' regions, but he found that the specimens, collected in India, having a *campestris*-like appearance, were actually of *A. barbirostris*. There is, however, need to look for this species very carefully in India, particularly in Assam and neighbouring hill states. In Malaysia, it is a highly anthropophilic species and is an

important vector of malaria and also of filariasis due to *B. malayi*. Its habits are very similar to those of *A. barbirostris*.

A. nivipes (Theobald), 1903

A member of the *Neocellis*, it is very much like *A. philippinensis* but can be distinguished. Reid, Wattal & Peters (1966) have studied this complex with special reference to India and no definite records of *A. nivipes* in India have been found. But according to Reid (1968) some references to *A. pallidus* in Assam and Burma could be referred to this species. The habits of adults and larvae are very similar to those of *A. philippinensis*. This is a species to look for eastern India, especially because of its vectorial potentialities. It is perhaps a minor human malaria vector in Malaysia.

A. vanus Walker, 1859

Wattal *et al.* (1962) have identified some specimens in India as *A. vanus*. But the main area of distribution of this species is Kalimantan, Philippines, Sulawesi, etc. far removed from north India and Reid (1965) and Harrison and Scanlon (1975) regarded it as a misidentification of *A. barbirostris*.

A. indefinitus (Ludlow), 1904

Long regarded as a variety of *A. subpictus*, it has been raised to the status of a species. It has a very wide distribution from Malaysia, to the Philippines and northwards to Taiwan. Reid (1968) thinks that the *A. subpictus* var. *vadakadiensis* Doraisamy, 1963 found near Rameswaram, Tamil Nadu, may acutally be *indefinitus*. This needs further study of the material from south Tamil Nadu. If confirmed, *A. indefinitus* can be stated to occur in India.

A. indefinitus can be distinguished from typical *A. subpictus* by (a) subapical dark band on female palpi shorter, giving the subapical band a shorter appearance (somewhat like *vagus* but not quite); (b) proboscis faintly pale towards tip; (c) larva with shorter palmate hair on abd. seg. 1 with leaflets (8 or more) with clearly differentiated filaments; (d) inner shoulder hair 14 or more branches (Reid, 1968).

The inner clypeal hair is bifid in about 20 per cent of larvae in Malaysia; most of them are bifid and sometimes trifid in Indonesia. Outer clypeals may also be branched. (Var. *vadakadiensis* has bifid inner clypeal hairs in most cases).

This species is largely a cattle feeder. It has no role of a malaria vector in Malaysia or the Philippines but has been regarded as a vector in south Java (Sunderaraman *et al.* 1957). Harinasuta *et al.* (1976) include *A. subpictus* as a vector in Indonesia (perhaps referring to *indefinitus*).

All the above species are included in the keys for identification in this volume, so that workers both in the western and eastern zones of the country may be able to distinguish them if found.

1. Anophelines of Afghanistan

References: 1. Ramachandra Rao (1951)

2. Iyengar (1954)
3. Dhir and Rahim (1957)
4. Polovov *et al.* (1975)
5. Onori *et al.* (1975)
6. Eshgy and Mushin (1978)

A. North of Hindukush Mountains, Palaearctic region:

A. claviger
A. hyrcanus pseudopictus
A. sinensis
A. superpictus
A. pulcherrimus

B. South of Hindukush Mountains, Oriental region, Indo-Pakistan Sub-region:

A. annularis
A. culicifacies
A. fluviatilis
A. nigerrimus
A. maculatus
A. moghulensis
A. multicolor
A. pulcherrimus
A. splendidus
A. stephensi
A. subpictus
A. superpictus
A. turkhudi
A. vagus

Malaria vectors:

A. North of the Hindukush: Ref: Dhir & Rahim, 1957 Onori *et al.*, 1975

A. hyrcanus (Var?)
A. pulcherrimus
A. sinensis
*A. superpictus**

* The first detection of sporozoites in this species in this area was made by the present author in 1950. These are included in Dhir & Rahim (1957).

B. South of the Hindukush: Ref: Ramachandra Rao, 1951 Dhir & Rahim, 1957

A. culicifacies
A. superpictus

NOTES:

1. 17 experts of the Martinovsky Institute of Tropical Medicine, Moscow, and Malaria Institute of North Afghanistan (Polovov *et al.*, 1975) made a study of the four distinct landscape epidemiological zones of the country and determined that 90 per cent of malaria cases occurred in a zone of irrigated rice fields. *A. pulcherrimus*

and *A. hyrcanus* were the vectors which were resistant to DDT and BHC, but they were still susceptible to organophosphorus insecticides. Larviciding with abate also seems to have given good results.

2. In the northeastern region of Afghanistan the study by Onori *et al.* (1975) found that *A. superpictus,* which was formerly considered to be the chief vector, had practically disappeared after DDT spraying commenced in 1950. Malaria transmission however occurred and *A. hyrcanus* and *A. pulcherrimus* were regarded to play a role in transmission. *A. hyrcanus* in this area is largely exophilic and exophagic and *A. pulcherrimus* partly so. In 1969 *A. hyrcanus* showed a high degree of resistance to DDT while *A. pulcherrimus* was susceptible but showed incipient resistance. Two members of the *A. hycranus* group had been found prior to the use of DDT, *viz., pseudopictus* and *sinensis.* In 1969 the latter had disappeared and only the former was present.

3. Eshghy and Mushin (1978) report a fairly high degree of resistance in *A. culicifacies* to DDT, a 4 per cent DDT paper giving kills of only 10.3 to 39.4 per cent in various parts of Afghanistan. In Laghman, in the southeastern part of Afghanistan bordering Pakistan, the kill was 39.4 per cent. Malaria has, consequently, increased in many places after about 15 years of good control.

2. Anophelines of Pakistan

References: Christophers (1933)
Aslamkhan (1971)

Subgenus Anopheles:

A. barbirostris
A. barianensis
A. nigerrimus
A. gigas
A. gigas var. *simlensis*
A. habibi
A. lindesayi

Subgenus Cellia :

A. annularis
A. culicifacies
A. dthali
A. fluviatilis
A. maculatus
A. maculatus var. *willmorei*
A. moghulensis
A. multicolor
A. pallidus

A. pulcherrimus
A. sergentii
A. splendidus
A. stephensi
A. stephensi var. *mysorensis*
A. subpictus
A. superpictus
A. theobaldi
A. turkhudi

NOTES :

1. The major malaria vectors in Pakistan are :

A. culicifacies
A. stephensi and var. *mysorensis*
A. superpictus and
probably *A. fluviatilis* in some places as it has been found to be a vector in Iran.

3. Anophelines of Nepal

References: Peters *et al.* 1955
Brydon *et al.* 1961
Joshi, *et al.* 1964
Shrestha 1966
Pradhan *et al.* 1971
Shrestha, Personal Communication (1979)

List as given by Shrestha is reproduced below:

Subgenus Anopheles :

A. aitkenii
*A. annandalei**
*A. barbirostris**
A. gigas
A. lindesayi
A. nigerrimus
*A. peditaeniatus**
*A. pseudosinensis**
*A. sinensis**

Subgenus Cellia :

A. aconitus
A. annularis
A. culicifacies
A. filipinae
A. fluviatilis
A. jamesii
A. minimus
A. pallidus
*A. philippinensis**
*A. ramsayi**
A. splendidus
*A. stephensi**

A. jeyporiensis
A. karwari
A. kochi
A. maculatus
and var. *willmorei*
A. majidi
*A. mangyanus**
A. subpictus
A. tessellatus
*A. theobaldi**
*A. turkhudi**
A. vagus
A. varuna

"In addition to these listed three more species were recorded during the I.B.D.C. period; *A. leucosphyrus, A. pulcherrimus* and *A. umbrosus*. Since these specimens are not found now and no others have been collected, these are considered as doubtful."

Comments: 1. Several species in the above list are not listed as occurring in Nepal in Knight and Stone (1977) and Stone and Delfinado (1973). Except for the species which are known to be very widespread, they have been marked with an asterisk. Most of them may occur in Nepal as they are found in the neighbouring regions of India. However doubt remains regarding species like *A. mangyanus* and *A. pseudosinensis*. Their occurrence needs further confirmation.

2. *A. annandalei* — It is not clear whether the record refers to *A. annandalei* or *A. interruptus* which has now been given a specific status. This needs confirmation.
3. *A. gigas* — Presumably it refers to *A. gigas* var. *simlensis* which is very common in the Himalayas. *A. gigas* type is reported to be confined to South India. However Shrestha says (Personal communication) that both occur in Nepal.
4. *A. maculatus* — Both *A. maculatus* and *A. maculatus* var. *willmorei* occur in Nepal.
5. *A. sinensis* — Needs confirmation. Shrestha records it in all divisions of Nepal.
6. *A. turkhudi* — This is the northern most record of this species in this sub-region.

Malaria vectors: *A. minimus, A. fluviatilis, A. maculatus willmorei* and *A. annularis* have been incriminated as vectors of malaria in Nepal. Pradhan *et al.* (1971) have also recorded *A. maculatus willmorei* as a vector in the Gum valley. Shrestha (Personal communication 1979) has found *A. annularis* with sporozoite positives in outer Terai, indicating that the species has a much wider sphere of influence than believed hitherto.

NOTES:

1. Peters *et al.* (1955) made a survey of the anophelines in the Rapti Valley area of the Terai Region of Nepal and determined *A. fluviatilis* as the vector. They detected the following species. *A. fluviatilis, A. culicifacies, A. minimus, A. macu-*

latus, A. splendidus, A. annularis, A. majidi, A. subpictus, A. vagus, A. varuna and *A. aitkeni.* The common species were *fluviatilis*—23 per cent; *culicifacies*—22 per cent; *maculatus*—17 per cent; and *splendidus*—29 per cent.

The following species were also found as larvae; *A. "hyrcanus", A. barbirostris, A. lindesayi* and *A. aconitus.* Only *A. fluviatilis* was found naturally infected (two specimens infected; 0.4 per cent) in the Chitwar Valley. Later in the same valley *A. minimus* was determined as the principal vector, and *A. fluviatilis* as a secondary vector. It may be noted that Issaris *et al.* (1953), in the neighbouring Terai region of Uttar Pradesh, found similar infection rates in *A. fluviatilis* between October and May. In other studies *A. minimus* has been found to be comparatively rare.

Anthropophilic index of *A. fluviatilis* was 21.4 per cent out of 113 tested and of *A. culicifacies* 12.7 per cent of 87 tested. Joshi *et al.* (1964) found that *A. kochi* was quite common.

2. Brydon *et al.* (1961) confirmed that *A. minimus* was the primary vector and *A. fluviatilis* the secondary vector in the forest belt of Terai and in the inner belt of Terai. In the hilly regions *A. fluviatilis* was the only vector.

3. In 1966 Shrestha again confirmed the distribution of *A. minimus* that it is restricted to the Inner Terai and *A. fluviatilis* was found infected at an elevation of 4300 feet (1290 metres).

4. An extensive survey was carried out in 1969 by Pradhan *et al.* (1971) in Khater and Gum Valley of Nepal leading to the following conclusions.

"Khater river valley (Alt: 3800-7000 feet) and Gum Valley (3500 to 10,400 ft.) were selected for the study of malaria transmission in hilly region. Along with entomological observations, malariometric surveys were carried out and parasite rate found to be 8.9% for Khater river valley and 3.40% for Gum valley. The entomological observations recorded: *A. fluviatilis, A. maculatus, A. maculatus* var. *willmorei, A. annularis, A. splendidus, A. vagus, A. culicifacies* and *A. lindesayi* from Khater river valley. The predominant species was *A. fluviatilis* followed by *A. maculatus.* The per man-hour density of *A. fluviatilis* was 17.61 for human sheds and 34.37 for animal sheds and the density per man per night was 2.60 to 14.00 during July. In the same month three *A. fluviatilis* (out of 2,540) were found to have sporozoites in their glands. From Gum Valley only five species of Anophelines—*A. fluviatilis, A. maculatus* var. *willmorei, A. maculatus, A. annularis* and *A. subpictus* were recorded. The predominant species was *A. maculatus* var. *willmorei* having a per man hour density of 9.98 for human sheds in August and 69.82 from animal sheds in September and the density per man per night was 8.88 in August. This species was incriminated by finding sporozoites in four specimens (three in August and one in first week of September) in 1,183 dissections."

The presence of *A. maculatus* var. *willmorei* was noted upto an elevation of 10,400 feet (3500 metre) above M.S.L. (in the Gum Valley). The absence of *A. minimus* in the hills is noteworthy. Their studies showed that both *A. minimus* and *A. fluviatilis* occurred in the lower regions, but only *A. fluviatilis* occurred in the hills.

The susceptibility tests with *A. fluviatilis* from Khater river valley and *A. macu-*

latus willmorei from Gum valley against DDT showed that both of them were susceptible. A few survivors at 4 per cent DDT concentration were however noted.

4. Anophelines of Bangladesh

References: Christophers (1933)
Aslamkhan (1971)
Rosenberg and Maheswari (1952)

Subgenus Anopheles:

A. barbirostris
A. gigas (see Note below)
A. nigerrimus
A. bengalensis
A. gigas var. *simlensis*
A. umbrosus

Subgenus Cellia:

A. aconitus
A. balabacensis (*A. dirus*?)
A. fluviatilis
A. jeyporiensis
A. kochi
A. maculatus var. *willmorei*
A. pallidus
A. ramsayi
A. stephensi
A. sundaicus
A. theobaldi
A. varuna
A. annularis
A. culicifacies
A. jamesii
A. karwari
A. maculatus
A. minimus
A. philippinensis
A. splendidus
A. subpictus
A. tessellatus
A. vagus

NOTES:

1. The malaria vectors in Bangladesh are the same as in West Bengal and Assam, *A. minimus, A. philippinensis* and *A. annularis*. The status of *A. sundaicus* is not clear. *A. balabacensis* (= *A. dirus*) is a vector in Sylhet District (Rosenberg and Maheswari, 1982).

2. In addition to the above the following species determined from the neighbouring regions of India may occur in parts of Bangladesh.

A. aitkenii
A. ahomi
A. crawfordi
A. interruptus
A. nitidus
A. roperi
A. argyropus
A. barbumbrosus
A. insulaeflorum
A. lindesayi
A. peditaeniatus
A. sinensis

3. The record of *A. gigas* is interesting. Hitherto it had been regarded as present in south India only. (See also under Nepal). Puri (1948) has shown a record in "Assam".

4. Even in their paper published in 1971, Khan and Talibi (1971) had pointed out that *A. minimus* was still a vector in the Chittagong Hill tracts of Bangladesh, with two peaks of transmission. How intense the transmission was can be judged by the

fact that nearly 52 per cent of infants when they were over two months old had their first infection.

5. Anophelines of Bhutan

Published or officially authenticated records of the anophelines of Bhutan are not available to the author.

6. Anophelines of Burma

References: Khin-Maung-Kyi (1971)
Stone and Delfinado (1973)
Stone and Knight (1977)

Subgenus Anopheles:

*A. aitkenii**
*A. argyropus**
A. gigas
*A. insulaeflorum**
*A. lindesayi**
A. peditaeniatus
*A. bengalensis**
A. barbirostris
A. nitidus
A. kyondawensis
A. nigerrimus
A. sinensis

Subgenus Cellia:

A. aconitus
A. balabacensis
A. fluviatilis
A. jeyporiensis
A. karwari
A. maculatus var. *willmorei**
A. minimus
A. pampani
A. ramsayi
A. stephensi
A. sundaicus
A. theobaldi
A. varuna
A. annularis
A. culicifacies
A. jamesii
A. jeyporiensis var. *candidiensis*
*A. kochi**
A. majidi
A. pallidus
A. philippinensis
A. splendidus
A. subpictus
A. tessellatus
A. vagus

Species marked with an asterisk (*) are not shown to occur in Burma by Stone and Delfinado (1973) but are listed by Khin-Maung-Kyi. *A. pampani* is shown by Knight and Stone (1977) but not by Khin-Maung-Kyi.

Certain other species found to occur both in India and Thailand which one may expect in Burma are: *A. barbumbrosus, A. crawfordi* and *A. roperi.*

NOTES:

References: Postiglione and Venkat Rao (1956)
Khin-Maung-Kyi (1971)
Harinasuta *et al.* (1976)

1. According to Postiglione and Venkat Rao (1956) the main malaria vector in Burma was *A. minimus* in the thickly wooded parts of northern hill range, but *A. leucosphyrus* (*balabacensis*) was also an important vector.
A. culicifacies was suspected to be a vector in the plains on the basis of one gland infected specimen found.
Other vectors are *A. annularis* in the Arakan border; and *A. jeyporiensis* (Type or variety?).
Regarding *A. sundaicus* they state that it is suspected on epidemiological grounds though no infected specimens have been found.

2. Khin-Maung-Kyi (1971) has provided more recent information. The major malaria vectors are:

A. hyrcanus group:	Probable secondary role and should not be ignored, particularly *A. sinensis.*
A. balabacensis:	A major vector during monsoon in the thickly forested areas of Burma.
A. culicifacies:	Responsible for local outbreaks. Many dissections with negative results have been made but a few positives have also been found.
A. minimus:	An important vector. Many positive dissection results in many localities.
A. jeyporiensis:	High infection rates found in both Indo-Burma border and China-Burma border; it should be regarded as a vector wherever it occurs in numbers.
A. annularis:	A secondary vector. Several reports of positive dissections.
A. sundaicus:	Suspected on epidemiological grounds.
A. philippinensis	Regarded as not of importance except purely locally in the Bhamo region.

3. Postiglione and Venkat Rao (1956) have summarized the dissection records in Burma as shown in Table 21.

4. According to Harinasuta *et al.* (1976) the malaria vectors are:

*A. minimus minimus**
A. annularis
A. sundaicus
A. culicifacies
A. balabacensis balabacensis

(*Important vector)

Table 21. Results of Dissections in Burma
(POSTIGLIONI AND VENKAT RAO, 1956)

Species	Number dissected	Gut +ve	Gland +ve	Infection rate percent	Sporozoite rate percent
*A. minimus**	3,283	50	29	1.5	0.9
A. "leucosphyrus"	1,296	?	12	?	1.0
*A. culicifacies**	303	2	—	0.97	—
*A. jeyporiensis***	161	—	—	—	—
A. maculatus	287	1	—	0.4	—
A. philippinensis	686	2	1	0.3	0.1

* Dissections of these two species carried out in Southern Shan States by U Tin (1928) and Singh (1940) are not included in this Table as gut and gland infections were not shown separately.

** Infections were, however, found in the Arakan region and Burma-Yunnan border by Robertson (1941) (quoted by Khin-Maung-Kyi, 1971).

7. Anophelines of Thailand

References : Peyton and Scanlon (1966)
Harrison and Scanlon (1975)
Knight and Stone (1977)
Stone and Delfinado (1973)

Subgenus Anopheles :

A. argyropus
A. asiaticus
A. barbirostris
A. bengalensis
A. campestris
A. donaldi
A. hodgkini
A. insulaeflorum
A. lesteri paraliae
A. montanus
A. palmatus
A. pollicaris
A. roperi
A. sinensis
A. tigertti
A. whartoni
A. aberrans
A. baezai
A. barbumbrosus
A. bukleyi
A. crawfordi
A. fragilis
A. nitidus
A. interruptus
A. letifer
A. nigerrimus
A. peditaeniatus
A. pursati
A. separatus
A. stricklandi
A. umbrosus

Subgenus Cellia :

A. aconitus
A. balabacensis var. *balabacensis*
A. annularis
A. balabacensis var. *introlatus*

A. culicifacies
A. hackeri
A. jamesii
A. jeyporiensis var. *candidiensis**
A. kochi
A. minimus
*A. pallidus**
A. philippinensis
A. ramsayi
A. splendidus
A. subpictus
A. tessellatus
A. dirus
A. indefinitus
*A. jeyporiensis**
A. karwari
A. maculatus
A. nevipes
A. pampanai
A. pujutensis
A. riparis var. *macarthuri*
A. stephensi
A. sundaicus
A. vagus

*NOTE : While Peyton and Scanlon say that *A. jeyporiensis* was a misidentification and do not mention *A. j. candidiensis*, Harrison and Scanlon (1975) include *A. jeyporiensis* in their keys but leave out *A. j. candidiensis*. Knight and Stone (1977) do not include Thailand in the geographic range of *A. jeyporiensis* but include *A. j. candidiensis*. Further clarification is needed. *A. pallidus* occurs in the keys of Peyton and Scanlon but not in the keys of Harrison and Scanlon. Knight and Stone include Thailand in the distribution of this species.

Doubtful records :

Subgenus Anopheles :

A. aitkenii
A. albotaeniatus
A. gigas formosus
A. gigas sumatrana

Subgenus Cellia :

A. filipinae
A. fluviatilis
A. maculatus var. *willmorei*
A. majidi
A. varuna

NOTE :

1. Malaria vectors in Thailand : According to Harinasuta *et al.* (1976) the malaria vectors in Thailand are:

A. minimus minimus
A. balabacensis balabacensis
A. aconitus
A. maculatus

] (Most important vectors.)

A. sundaicus
A. campestris
A. philippinensis

2. The recent work of Ismail *et al.* (1974) and others show that *A. minimus* which has practically disappeared in large parts of India, still persists in Thailand and is a vector along with *A. b. balabacensis.* Regarding the new species, *A. dirus,* see under *A. balabacensis.*

8. Anophelines of Malaysia

It should be noted that the modern Malaysia includes not only the mainland but also parts of N. Borneo including Sabah and Sarawak. It is desirable to give separate lists for these two areas.

References : Knight & Stone (1977)
Stone and Delfinado (1973)
Reid (1968)

	Malaysia mainland	N. Borneo
	+=present;	O=absent
Subgenus Anopheles :		
A. aitkenii	+	+
A. acaci	O	+
A. albotaeniatus	+	+
A. argyropus	+	O
A. asiaticus	+	O
A. barbirostris	+	O
A. barbumbrosus	+	O
A. bengalensis	+	+
A. baezai	+	+
A. barianensis	O	+
A. brevipalpis	+	+
A. brevirostris	+	O
A. campestris	+	O
A. collessi	+	+
A. crawfordi	+	O
A. donaldi	+	+
A. fragilis	+	+
A. gigas	O	+
A. hodgkini	+	+
A. hunteri	+	+
A. nitidus	+	+
A. insulaeflorum	+	O

A. interruptus	+	O
A. lindesayi	+	O
A. lesteri	+	+
A. letifer	+	+
A. mantanus	+	+
A. nigerrimus	+	+
A. noviae	+	O
A. palmatus	+	+
A. pollicaris	+	O
A. peditaeniatus	+	+
A. roperi	+	+
A. separatus	+	+
A. similissimus	+	+
A. sinensis	+	O
A. sintonoides	+	O
A. stricklandi	+	O
A. umbrosus	+	+
A. vanus	O	+
A. wellingtonianus	+	O
A. watsoni	+	O
A. whartoni	+	+

Subgenus Cellia :

A. aconitus	+	+
A. aurirostris	+	O
A. balabacensis	+	+
A. bakeri	+	+
A. indefinitus	+	O
A. kochi	+	+
A. leucosphyrus	+	+
A. minimus minimus	+	+
A. pujutensis	+	+
A. riparis	+	+
A. saungi	O	+
A. stookesi	O	+
A. subpictus	+	O
A. sundaicus	+	+
A. tessellatus	+	+
A. vagus	+	+

NOTES :

1. Malaria vectors :

According to Harinasuta *et al.* (1976), the malaria vectors in Malaysia are:

A. maculatus	(Important Vector)
A. sundaicus	"
A. campestris	"
A. balabacensis balabacensis	
A. letifer	

2. Reviewing the malaria vectors in Malaysia, Sandosham (1962) stated as follows :

A. maculatus is a vector found in the hill areas. *A. letifer (umbrosus* group) and *A. campestris* (*barbirostris* group) in the coastal plains, *A. sundaicus* along the coast, *A. nigerrimus* occasionally in the plains and *A. balabacensis* and *A. leucosphyrus* in the hilly and forested areas. *A. umbrosus* has long been regarded as a vector but early records may probably refer to *A. letifer.* It is also possible that many *umbrosus* group infections may be due to mouse-deer malaria.

3. Wharton (1962) reviewing the malaria vector problems in South East Asia regarded the two main ones to be (1) exophily of *A. balabacensis,* and (2) the insecticidal resistance in *A. sundaicus,* the minor ones being the problem of secondary vectors and monkey-malaria. Information about the vectors of monkey-malaria would be useful so that the infections found in mosquitoes may not be confused with those of human malaria.

The *leucosphyrus* group consist of 13 forms, all being forest mosquitoes, of which *A. balabacensis* is the most important vector in the monsoon countries north of Malaysia and in north Borneo (Sarawak and Sabah), and *A. hackeri* in southern Borneo (Kalimantan).

In the *barbirostris* group there are 11 species of which six occur in Malaysia. *A. barbirostris,* the commonest of them, is zoophilic and harmless. *A. campestris* is important as a vector in the coastal plains of Malaysia and is anthropophilic. *A. donaldi* is the main vector in Borneo (Sarawak and Sabah).

In the *hyrcanus* group there are 8 species in Malaysia. *A. nigerrimus* is the only vector in Malaysia and parts of Indonesia. *A. sinensis* is not a vector in South East Asia though it was regarded so in China, *A. lesteri* rather than *A. sinensis sensu stricto* is nowadays regarded as the vector. In the *umbrosus* group, 9 species occur in Malaysia, a number of which have been found naturally infected with oocysts and sporozoites. Now they are regarded to be of non-human origin. *A. letifer* is an important human malaria vector but the name covers more than one species. Quite a few high infection rates have been found in *A. umbrosus* and *A. baezai,* but the infections are perhaps of non-human plasmodia.

4. Reid (1968), summarizing the observations of many workers has shown that as many as 17 species have been found infected in nature. But the most important ones are those with records of over 1.0 per cent sporozoite rates:—

A. peditaeniatus	2.3 per cent
A. baezai	2.0 "
A. roperi	5.2 "
A. umbrosus	1.7 "

A. maculatus	1.4	*per cent*
A. balabacensis	10.0	"
A. balabacensis introlatus	2.0	"

9. Anophelines of "Borneo"

Borneo of McArthur includes both N. Borneo now included in Malaysia and Kalimantan now included in Indonesia.

According to McArthur (1950a) 42 species of *Anopheles* were recorded in Borneo; 16 have been dissected and 9 were recorded as infected with malaria parasites. The important vectors were *A. leucosphyrus, A. umbrosus, A. sundaicus* and *A. baezai* in order of importance. The infections found but regarded as of less importance were those in *A. separatus, A. "hyrcanus", A. barbirostris, A. tessellatus* and *A. minimus* variety *flavirostris.* Further study is needed about them.

A. maculatus, long regarded to be the chief vector of malaria in Borneo, as in the interior of Malaysia, has not given any evidence of being actually concerned with the transmission in Borneo.

The 42 species are :

Series Anopheles :

A. aitkenii aitkenii
A. aitkenii bengalensis
A. aitkenii borneensis
A. aitkenii palmatus
A. brevipalpis
A. gigas sumatrana

Series Myzorhynchus :

A. albotaeniatus
A. barbirostris
A. barbumbrosus
A. "hyrcanus"
A. hyrcanus sub. sp. near *sinensis*
A. novumbrosus
A. sinensis
A. vanus
A. baezai
A. barbirostris var. *pallida*
A. hunteri
A. nigerrimus
A. letifer
A. montanus
A. separatus
A. umbrosus

Series Lophoscelomyia :

A. asiaticus

Subgenus Cellia :

Series : Neomyzomyia

A. hackeri
A. leucosphyrus var. *balabacensis*
A. kochi
A. leucosphyrus
A. punctulatus

A. leucosphyrus var. *pujutensis*
A. tessellatus
A. watsoni

Series Myzomyia :

A. aconitus
A. minimus
A. minimus var. *flavirostris*

Series Neocellia :

A. annularis
A. errabundus
A. karwari
A. maculatus
A. philippinensis

Series Pyretophorus :

A. litoralis
A. ludlowi
A. subpictus
A. subpictus var. *malayensis*
A. sundaicus
A. vagus
A. vagus var. *limosus*

NOTES :

1. The above nomenclature was that prevalent before the work of Colless, Reid and others. The names *"hyrcanus"* and *"leucosphyrus"* include several species.
2. Among vectors, the true status of *A. umbrosus* is in doubt (See under *A. umbrosus*).
3. See Reid (1968) for the fauna of North Borneo.

10. Anophelines of Vietnam

Reference : Knight & Stone (1977)
Stone and Delfinado (1973)
Pham Quong Tuan (1973)

Subgenus Anopheles :

A. aitkenii
A. baezai
A. campestris
A. nitidus
A. lesteri
A. peditaeniatus
A. argyropus
A. barbirostris
A. crawfordi
A. letifer
A. nigerrimus
A. sinensis

Subgenus Cellia :

A. aconitus
A. balabacensis
A. jeyporiensis var. *candidiensis*
A. kochi
A. philippinensis
A. subpictus
A. tessellatus
A. annularis
A. jeyporiensis
A. karwari
A. minimus minimus
A. splendidus
A. sundaicus
A. vagus

NOTES :

Malaria vectors:

1. According to Harinasuta *et al.* (1976)

A. minimus var. *minimus*
A. balabacensis balabacensis
A. jeyporiensis
A. sundaicus
A. maculatus

(Important vectors)

2. According to Pham-Quang-Tuan (1973)

Principal vectors :

A. minimus var. *minimus*
A. balabacensis
A. jeyporiensis
A. jeyporiensis var. *candidiensis*
A. sundaicus

In high plateau mountainous jungle-covered areas :

A. jeyporiensis
A. jeyporiensis candidiensis
A. minimus minimus
A. balabacensis

In coastal plains and delta :

A. sundaicus

Several other species are suspected on epidemiological grounds, such as: *A. maculatus* in high plateau and *A. subpictus, A. minimus, A. tessellatus* and *A. barbirostris* in other areas.

11. Anophelines of Laos

References : Knight and Stone (1977)
Stone and Delfinado (1973)

The following list is compiled from the above two publications. Most of the species recorded are those which are also found in Kampuchea and Vietnam.

Subgenus Anopheles :

A. baezai
A. bengalensis
A. interruptus
A. nitidus
A. sinensis
A. barbirostris
A. gigas baileyi?
A. nigerrimus
A. peditaeniatus

Subgenus Cellia :

A. aconitus
A. balabacensis balabacensis
A. fluviatilis
A. jamesii
A. karwari
A. maculatus
A. pallidus
A. ramsayi
A. subpictus?
A. tessellatus
A. annularis
A. culicifacies?
A. indefinitus
A. jeyporiensis
A. kochi
A. minimus minimus
A. philippinensis
A. splendidus
A. sundaicus
A. vagus

NOTES :

1. The list is perhaps incomplete because several species which occur both in Kampuchea and Vietnam do not find a place in it.
2. According to Harinasuta *et al.* (1976), the malaria vectors in Laos are :—

A. minimus minimus
A. balabacensis balabacensis

12. Anophelines of Kampuchea (Cambodia) (Also formerly known as Khmer Republic)

References : Harrison and Klein (1975)

Subgenus Anopheles :

*A. argyropus**
A. barbirostris
A. bengalensis
A. crawfordi
A. insulaeflorum
*A. letifer**
A. nitidus
A. pursati
*A. separatus**
A. whartoni
*A. baezai**
*A. barbumbrosus**
A. campestris
A. hodgkini
A. interruptus
*A. nigerrimus**
A. peditaeniatus
*A. roperi**
A. sinensis

* These are new records established by surveys by Klein.

Subgenus Cellia :

A. aconitus
A. balabacensis
A. indefinitus
A. karwari
A. maculatus
A. pampanai
A. ramsayi
A. subpictus
A. tessellatus
A. annularis
A. fluviatilis
A. jeyporiensis
A. kochi
A. minimus minimus
A. philippinensis
A. splendidus
A. sundaicus
A. vagus

NOTES :

1. The malaria vectors according to Harinasuta, Gilles and Sandosham (1976) are:

*A. minimus minimus**
A. balabacensis balabacensis
A. maculatus
A. sundaicus

* Important vector.

According to a map given by Yit Kim Seng (1973) in *Vector Control in South East Asia, A. maculatus* is a vector only in the two southern maritime districts *viz.*, Kom Kang and Kampli. The three other vectors are distributed throughout the country. *A. minimus* and *A. sundaicus* had been greatly affected by DDT spraying operations but not *A. balabacensis.*

13. Anophelines of Indonesia

Indonesia is a vast country consisting of over 3,000 islands, a few such as Sumatra, Java, Kalimantan and Sulawesi being very large, and many not bigger than a few square miles in size. In surface area the country exceeds in size any other south-east Asian country except India. The country extends over thousands of miles east to west and includes parts some of which occur in the Oriental region and others in the Australasian region. The major islands are Sumatra, Java, Kalimantan, (Borneo), Sulawesi (Celebes) and Timor in the Oriental Region and West Irian (formerly West New Guinea), the Moluccas and Ceram, all now part of Indonesia, but actually lying to the east of the Weber's line and belonging to the Australasian region.

In preparing the following list of the anopheline fauna of the country in the oriental region reliance has been placed on the catalogues by Stone and Delfinado (1973) and Knight and Stone (1977). The book by Bonne-Wepster and Swellengrebel (1953) on the *Anopheline Mosquitoes of the Indo-Australian Region* has also been useful.

There have been several changes in place names which sometimes leads to confusion. For example the name 'Borneo' used by Knight and Stone could include both

Kalimantan of Indonesia and Sarawak, Sabah and Brunei of North Borneo. Therefore 'Borneo' in the following lists may include both regions. The fauna of 'Borneo' following McArthur has been dealt with separately as also that of the Malaysian part of the island.

Subgenus Anopheles:

A. acaci	'Borneo'
A. aitkenii	
A. albotaeniatus	
A. annandalei	Java
A. argyropus	Sumatra, Java
A. baezai	
A. bancroftii var. *barbiventris*	Sulawesi
A. barbirostris	
A. barbumbrosus	
A. borneensis	'Borneo'
A. brevipalpis	'Borneo'
A. collessi	'Borneo'
A. crawfordi	Sumatra
A. donaldi	
A. fragilis	
A. gigas	Sulawesi
A. gigas var. *crockeri*	'Borneo'
A. g. var. *danaubendo*	Sumatra
A. g. var. *oedjalkalah*	Sumatra
A. g. var. *pantjarbatu*	Sumatra
A. g. var. *sumatrana*	Sumatra
A. hunteri	Sumatra
A. indiensis	Sumatra, 'Borneo'
A. insulaeflorum	
A. lesteri sub sp. *paraliae*	'Borneo'
A. letifer	Sumatra, 'Borneo'
A. montanus	Sumatra, 'Borneo'
A. nigerrimus	Java
A. nitidus	Sumatra
A. palmatus	Java
A. peditaeniatus	
A. pseudobarbirostris	Sulawesi
A. roperi	Sumatra, 'Borneo'
A. separatus	Sumatra, 'Borneo'
A. similissimus	Sumatra
A. sinensis	Sumatra
A. umbrosus	
A. vanus	Sulawesi

Subgenus Cellia :

A. aconitus	
A. annularis	
A. balabacensis	Java, 'Borneo'
A. hackeri	Sumatra, 'Borneo'
A. indefinitus	Sulawesi
A. karwari	
A. ludlowi	
var. *torakala*	Sulawesi
A. leucosphyrus	Sumatra, 'Borneo'
A. maculatus	
A. minimus	
var. *flavirostris*	Java, Sumatra, 'Borneo'
A. pallidus	
A. parangensis	Sumatra, 'Borneo'
A. philippinensis	
A. pujatensis	Sumatra, 'Borneo'
A. ramsayi	Sumatra, Java
A. riparis sub. sp. *macarthuri*	'Borneo'
A. saungi	'Borneo'
A. schueffneri	Sumatra, Java
A. subpictus	
A. stookesi	'Borneo'
A. sulawesi	Sulawesi
A. sundaicus	
A. tessellatus	
A. t. var. *kalaware*	
A. t. var. *orientalis*	Sulawesi, Java, Moluccas
A. vagus	
A. v. var. *limosus*	'Borneo'

NOTES:

1. Though only a few place names have been mentioned, most of the common species occur throughout Indonesia, and separate lists for each island has not been attempted.

2. The easternmost parts of Indonesia lying within the Australasian region, such as West Irian (Irian Batar), the Moluccas, Ceram etc., have a totally different faunal composition. It is a transition zone with members from both regions. Australasian species such as also occur in New Guinea are:

A. annulipes	*A. bancrofti*
A. annulatus	*A. cristatus*
A. incognitus	*A. koliensis*
A. meraukensis	*A. punctulatus* and *A. novaguinensis*

Several oriental species overflow into this area.

Malaria vectors:

According to Harinasuta *et al.* (1976) the malaria vectors in Indonesia are:

A. aconitus
important vector
(Java and Bali)
A. subpictus
A. nigerrimus
A. vagus
A. sundaicus
important vector
(Java and Bali)
A. maculatus
A. tessellatus

Resistance to DDT has been reported in *A. aconitus* in Central Java and Djakarta and is spreading to East Java. Resistance has also been found in *A. sundaicus* but the species has now disappeared from the north coast of Java and in the southern coast the species has reverted to susceptibility.

In that part of Indonesia which lies in the Australasian region infections have been found in (Russell *et al.*, 1963):

A. bancrofti
A. karwari
A. subpictus
A. amictus var. *hilli*
A. farauti
A. tessellatus
A. punctulatus

14. Anophelines of Singapore

The rural areas of Singapore have an anopheline fauna similar to that in the adjoining areas of Malaysia.

The malaria vectors in Singapore, are *A. maculatus* and *A. sundaicus*. The former is a major vector and the latter is a suspected vector.

15. Anophelines of Southern China (Including Tibet)

References : Russell, Rozeboom and Stone (1943)
Knight and Stone (1977)
Stone and Delfinado (1973)

Subgenus Anopheles :

A. aitkenii
A. interruptus
A. gigas bailey
A. insulaeflorum
(in Taiwan only)
A. kweiyangensis
A. lesteri
A. lindesayi ssp *pleccau?*
A. peditaeniatus
A. sineroides
A. aitkenii bengalensis
A. barbirostris
A. freyi
A. koreicus (=*edwardsi* of Yamada)
A. lindesayi ssp *japonicus?*
A. nigerrimus
A. sinensis
A. sintonoides

Subgenus Cellia :

A. annularis
A. fluviatilis
A. jamesii
A. karwari
A. maculatus
A. pattoni
A. stephensi
A. sundaicus
A. vagus
A. culicifacies
A. indefinitus
A. jeyporiensis var. *candidiensis*
A. kochi
A. minimus
A. philippinensis
A. subpictus
A. tessellatus

NOTES :

1. No recent original records have been available to the author except Knight and Stone's (1977) Catalogue. Whether the following species occur in South China needs clarification.

A. aconitus
A. atroparvus
A. balabacensis, (at least in the border of Vietnam, Laos and Burma.)
A. gigas simlensis, (at least in Tibet)
A. hyrcanus
A. sacharovi

2. The species which occur in Tibet only are not clear.

NOTES :

References: Kung Chien Chang *et al.* (1976) Anon. (1976)

In a recent report of anti-malaria activities in the Yunnan Province (*Chinese Medical Journal,* 1976, July pp. 257-263), 24 species of *Anopheles* were found in surveys made in 1958-59. The most widespread species *A. minimus* accounted for 71.6 per cent of all the anophelines collected in human dwellings. 5,802 females of *A. minimus* were dissected with a sporozoite rate of 0.4 per cent. 2,577 females of 8 other species were dissected and no infections were found. The infected *minimus* were found throughout the year, except in February. There were two peaks one in July and another in October. By 1962, the disease had been well controlled, mainly by anti-larval measures, using extracts of indigenous plants and HCH (666) on ponds and canals. Indoor residual spraying with HCH was also undertaken by barefoot doctors.

In the 1972-75 surveillance, 4,362 anophelines were collected, but only 26 *A. minimus* adults were found. This shows that there has obviously been a great reduction in the prevalence of *A. minimus* in South China also in recent years.

16. Anophelines of Sri Lanka

References : Carter (1950)
Rajendram and Jayawickreme (1951)

Harrison *et al.* (1974) have examined an extensive collection of mosquitoes from Sri Lanka. They have added one new species *A. reidi* to the previous list and a few new records.

Subgenus Anopheles :

A. aitkenii
A. barbirostris
A. gigas refutans
A. nigerrimus
A. reidi
A. barbumbrosus (reported by Reid 1962)
A. insulaeflorum
A. interruptus
A. peditaeniatus

Subgenus Cellia :

A. aconitus
A. culicifacies
A. ramsayi
A. maculatus
A. elegans
A. subpictus
A. vagus
A. annularis
A. jamesii
A. karwari
A. balabacensis
A. pallidus
A. tessellatus
A. varuna

NOTE:

Also one unidentified specimen of a member of *A. minimus* group.

A. pseudobarbirostris mentioned by Rajendram and Jayawickreme (1951) is now regarded as a misidentification.

NOTES:

1. Harrison *et al.* (1974) do not list the following species:

A. insulaeflorum
A. interruptus
A. culicifacies
A. ramsayi

Presumably they were not in the collections they saw. The absence of *A. culicifacies*, the principal malaria vector in their list is inexplicable.

2. The only known malaria vector is *A. culicifacies*.

17. Anophelines of Maldives

Only two species of *Anopheles* were detected by Iyengar *et al.* (1953) *viz.*, *A. tessellatus* and *A. subpictus*. The latter was rare. *A. tessellatus* was found in all the inhabited islands. It was breeding extensively in wells—shallow ones about a metre in diameter, with subsoil water about 1.0 to 1.3 metres below ground level. *A. tessellatus* was the vector in the islands. One out of 22 females examined had sporozoites. Previously, Covell (1944) had reported two gland and four gut infections among 160 specimens dissected in some of the islands. More recent reports indicate that *A. tessellatus* has disappeared from the islands and that *A. subpictus* is now regarded as the vector.

PART II

SPECIAL SECTION
INDIVIDUAL SPECIES

13

Individual Species

Detailed information on the biology of all the 51 individual species and their subspecies and varieties, which occur in India, is provided in this chapter. The order of treatment of the several aspects followed is: Name, Type locality, Location of type, Taxonomy, Distinguishing characters, Distribution, Prevalence, Adult bionomics, Larval ecology, Relation to disease and Control (only in the case of a few selected major vectors). In case of rare or uncommon species, the treatment is less extensive.

The order in which the species are arranged is given below. As far as possible species having close affinities are grouped together except in series *Neocellia* in which the 12 species are arranged alphabetically.

A. Subgenus Anopheles :

(i) Series Anopheles

aitkenii
pinjaurensis
culiciformis
barianensis
gigas and
var. *simlensis* and
var. *baileyi*
bengalensis
insulaeflorum
sintoni
lindesayi and spp. *nilgiricus*

(ii) Series Lophoscelomyia

annandalei
interruptus

(iii) Series Myzorhynchus

"hyrcanus" group
Argyropus
crawfordi
nigerrimus
nitidus
peditaeniatus
sinensis

"barbirostris" group
ahomi
barbirostris
barbumbrosus

"umbrosus" group
roperi
umbrosus

B. Subgenus Cellia:

(i) Neomyzomyia series

balabacensis

elegans
kochi
tessellatus

(ii) Myzomyia series

aconitus
fluviatilis
minimus
varuna
culicifacies
dthali
jeyporiensis and
var. *candidiensis*
majidi

(iii) Pseudomyzomyia series (Pyretophorus)

subpictus
and var. *vadakadiensis*
vagus
sundaicus

(iv) Paramyzomyia series

multicolor
turkhudi

(v) Neocellia series

annularis
karwari
moghulensis
philippinensis
ramsayi
stephensi and
var. *mysorensis*
jamesii
maculatus and var. *willmorei*
pallidus
pulcherrimus
splendidus
theobaldi

Total 51 species, 1 subspecies and 6 varieties.

Anopheles aitkenii James, 1903

Type locality: Karwar, Karnataka (formerly Bombay State).
Type: British Museum of Natural History, London.
Taxonomy: Harrison and Scanlon (1975) recognise 12 species in the *aitkenii* species group. They are: *aberrans* Harrison and Scanlon, Thailand; *acaci* Baisas, "Borneo" and the Philippines; *aitkenii* James (see below); *bengalensis* Puri, Oriental region, India and east up to the Philippines; *borneensis* MacArthur, 'Borneo'; *fragilis* (Theobald): Burma to Sulawesi; *insulaeflorum* (Swell and S. degraff). India and eastwards; *palmatus* (Rodenwaldt), Thailand and eastwards (not in the Philippines or Sulawesi); *pilinotum* Harrison and Scanlon, Philippines and Indonesia; *pinjau-*

rensis Barraud, India; *stricklandi* Reid, Malaysia; and *tigertti* Scanlon and Peyton, Thailand.

Among them only *aitkenii, bengalensis, pinjaurensis* and *insulaeflorum* occur in India. Indian workers should carefully examine the material they collect with the help of associated larvae and pupal skins. Reid (1965, 1968) regarded *A. aitkenii sensu stricto* to have a wide distribution as given below, but Harrison and Scanlon (1975) felt that it is restricted only to the Indian sub-region. They considered, based on larval chaetotaxy, that all records in other southeast Asian countries to refer to the other species of the group particularly *A. bengalensis.* The keys to the four members of the *aitkenii* group found in India are given below:

1. Phallosome very long, tubular, and expanded at the end. . . . . *pinjaurensis*
Phallosome shorter, not expanded at the end 2

2. Phallosome with some fine spinous projections laterally towards apex. Larva with IC simple, arising close together, with developed palmate hair on I; lateral hair III only half as stout as I and II, and carrying only 5-8 br. Outer part of dorsal lobe of harpago usually with three spines .... *insulaeflorum*
Phallosome without any spicular projections. Larva with IC bifurcate or branched, without developed palmate hair on I; lateral hair III as on previous segments 3

3. Larva with IC bifurcate at about 1/4 from base; outer part of dorsal lobe of harpago usually with three spines .... *aitkenii*
Larva with IC split into 3-5 br. about 1/2 from base; outer part of dorsal lobe of harpago usually with two spines .... *bengalensis*

Distinguishing characters: A brown small sized unornamented mosquito with a *Culex* like resting posture. Wings entirely dark; hind femur with distinct white spots at distal end; narrow rod-like scales on head. These characters distinguish the species group from the related *barianensis* and *culiciformis.* From *bengalensis* and *insulaeflorum* it can be distinguished by larval characters especially the form of inner clypeal hairs, and male hypophygia. See under those species.

Distribution: India, Nepal, Sri Lanka, Bangladesh, Burma, Thailand, China, Malaysia, West Irian, Indonesia, and Philippines.

In India: it occurs in an extensive range in the southern and eastern regions extending from Kerala through Tamil Nadu, Karnataka, Maharashtra, Madhya Pradesh, West Bengal to Assam, Meghalaya and Arunachal Pradesh. Perhaps it occurs in Bihar and Orissa too. Also occurs in the Andamans.

Ecology: Generally prevalent at higher altitudes upto 2000 metres, it is a species of the jungle and forested areas and very rarely found to enter houses.

Christophers (1933) records collecting a number of adults in the shade of the jungle attempting to bite man. In Taiwan the adults do not seem to be attracted to man even if they are abundant (Onori, 1942). It is more often collected in the larval form. Brooke Worth (1953) collected 10 adults and 460 larvae in the Western Ghats of Karnataka State and found that it had a seasonal cycle during the months of December and January.

Larvae have been found in a variety of breeding places. Small pools and seepages

in the jungle and tea garden drains which are shaded are the usual breeding places in Assam and neighbouring areas. It has been found in shaded pools close to hill streams and "peat-swamps". In the Nilgiris in the south, it is found in swamps, irrigation channels, rivers, rock pools and wells. The present author has collected larvae in arecanut garden drains where they are common in North Kanara District but no adults. Larvae are quite often found in flowing waters, even if rapid, in Malaysia, Indonesia and 'Indochina' (Hacker, 1919; Toumanoff, 1931, quoted by Christophers, 1933).

Margins of streams heavily shaded by the trees seem to be selected in Taiwan (Onori, 1942).

Relation to disease: No information. Unlikely to have any significance in human disease.

Anopheles bengalensis Puri, 1950

Type locality: Marianbarie (near Sukna), West Bengal.

Type: Paratype in N.I.C.D., Delhi (confirmed by Dr. B.L. Wattal). According to Harrison and Scanlon (1975) specimens deposited by Puri in the British Museum have not been found (confirmed by Dr. G.B. White in personal correspondence).

Taxonomy: Christophers (1933) pointed out the difficulty of identifying the adults of *A. aitkenii* and *A. bengalensis* from *A. insulaeflorum* on the basis of adult characters and stated that main reliance has to be on larval chaetotaxy (see under *A. aitkenii*). It was regarded as a variety of *A. aitkenii* but Reid (1965) raised it to the status of a species. This species is very closely related to *A. fragilis* (not occurring in India) from which it can be distinguished with difficulty.

Distinguishing characters: It can be distinguished in the larval and pupal stages and on male genitalia—

Adult: Outer part of dorsal lobe of harpago usually with 2 external spines and a club formed of two spines.

Larva: Inner clypeal hairs dividing into three at about 1/3 or more of their length from the base, the central branch usually dividing again commonly giving 4-7 branches.

Distribution: India, Bangladesh, Burma, Thailand, Malaysia, Philippines, Japan (Amami Island) and Vietnam. Knight and Stone (1977) include South China, Taiwan and Ryukyu Islands. In Burma, Khin-Maung-Kyi (1971) has reported the species from places including Falam area bordering India, but many specimens may be *A. aitkenii* (Harrison and Scanlon, 1975).

In India: Originally recorded from Bengal and Assam. Puri (1948) recorded it from Visakhapatnam, Andhra Pradesh. Rama Rao and Achuthan (1964) have recorded a specimen of larva from Coorg District (Karnataka), in a slow running stream along with *A. aitkenii*. Ramachandra Rao *et al.* (1973) and Bhat (1975a) recorded *A. bengalensis* from Darjeeling District at Tashiding (500 m). Bhat reared 3 males and 3 females from larvae.

Bionomics/Ecology: This species is usually found at altitudes between 500 m and

2000 m. Most information is based on larval collections only and very little information is available on adults. Descriptions of adults have been based on specimens reared from larvae. *A. bengalensis* is definitely a forest loving species and the breeding takes place well away from human habitations. There are some records of female *aitkenii* or *bengalensis* biting man in Thailand (Scanlon and Esah, 1965), and in Malaysia (MacDonald and Traub, 1960). The records, however, may relate to other species of the *aitkenii* group.

The larvae occur in seepage springs or slow running streams in which the water is clear. Dead leaves and floating debris of vegetation are normally found in places where the larvae are found. Bhat (*loc. cit.*) collected larvae from isolated pools along a stream. In Thailand larvae have been collected from all types of forests and bamboo grooves, sometimes near villages. In common with other forest inhabiting species, footprints of animals are also selected for breeding. *A. aitkenii* as well as *A. bengalensis* have very similar breeding habits. Harrison and Scanlon recorded infections of *Coelomomyces* in larvae in Thailand.

Relation to disease: No information.

Anopheles pinjaurensis Barraud, 1932

Type locality: Pinjaur, near Kalka, Punjab, India.

Type: National Institute of Communicable Diseases, Delhi.

Taxonomy: Barraud named it as a variety of *aitkenii*, but Christophers (1933) raised it to the status of a species.

Distribution: India. Known only from the type locality and from a single male.

Bionomics, etc : No information.

Anopheles insulaeflorum (Swellengrebel and Swellengrebel deGraff, 1920)

Type locality: Noesa, Kambangam (South Java, Indonesia).

Type: Unknown. However, Harrison and Scanlon (1975) have examined two larvae from a locality only 50 kms from the type locality. According to them, previous records from Ambon, Ceram, Philippines, Sulawesi, Lesser Sunda Islands, and Moluccas need re-examination.

Taxonomy: In India, the three species with which it is likely to be confused are *A. aitkenii, A. bengalensis* and *A. pinjaurensis.* Reid (1965 and 1968) attempted a revision of the entire group.

Distinguishing characters: Adult: A small sized mosquito with a *Culex* like posture. Head scales long and narrow, as in all members of the *aitkenii* group; all abdominal segments of the same colour; area of mesothorax anterior to scutellum with short fine setae unlike in *A. bengalensis* in which it is bare; in the closely related *aberrans* and *palmatus* (neither occurring in India) abdominal segments 4 and 5 in the former and segment 5 in the latter are generally much paler than other segments. Larvae can be distinguished from *A. bengalensis* and *A. aitkenii* by the

clypeal hairs, unbranched and close together.

Distribution: India, Nepal, Sri Lanka, Burma, Thailand, Malaysia, Philippines, Indonesia, Kampuchea, Vietnam and Taiwan. Also perhaps Bangladesh.

In India: Formerly recorded from two far removed places *viz.*, North Kanara and Coorg Districts (Karnataka) and Darjeeling District (West Bengal). After Puri's initial records (1930), larval collections were made by the present author between 1942 and 1946 in North Kanara, by Rama Rao and Achuthan (1964) in Coorg District and by Brooke Worth (1953) from Hassan District of Karnataka. Puri (1948) recorded it from Malabar (Kerala) and also Sibsagar District Assam. It occurs all along the Western Ghats of Karnataka.

Bionomics/Ecology: The habits of this species are known only as regards larvae. This is a forest species. The larval habitats of the species are ground pools, rock pools, streams, stream margins, arecanut garden trenches, seepage pools, etc., all in shade. As is usual with such breeding places in the forest shade, they are likely to be covered by rotting dead leaves and vegetation. Brooke Worth (1953) has found a few larvae in the Western Ghats area in Karnataka along edges of streams in the open, not far from larval habitats of *A. fluviatilis* and *A. varuna*.

Relation to disease: No information. Unlikely to be of any significance.

Anopheles culiciformis Cogill, 1903

Type locality: Karwar, Karnataka, India.

Type: British Museum of Natural History, London.

Taxonomy: Very difficult to distinguish from *A. sintoni* in the adult stage. Five species are recognised in this group:

alongensis Venhuis, *culiciformis* Cogill, *kyondawensis* Abraham, *sintoni* Puri and *sintonoides* Ho.

The only two members of this group occurring in India are *A. culiciformis* and *A. sintoni*.

Distinguishing characters: A medium sized mosquito, dark brown complexion and a culicine attitude while resting. Distinguished from *A. sintoni* mainly in the adult phallosome and in the larval characters. From *A. aitkenii* and *A. insulaeflorum* it can be distinguished by normal head scales and more shaggy palps.

All members of the *A. culiciformis* group are completely dark species with no pale scaling either on wings, palps or legs. All members of this group can also be recognised by the resting attitude resembling culicines. It would be desirable for Indian workers to be on the lookout for other related species.

Distribution: Known in India only. North Kanara (Karnataka), Goa, Savantwadi (Ratnagiri District, Maharashtra), Nilgiris (Tamil Nadu) and Malabar (Kerala). Record from Nilgiris is regarded doubtful by Covell (1927b).

It is interesting that Brooke Worth (1953) did not collect even a single specimen in his exhaustive studies in Hassan District in the Western Ghats about 200 kms south of North Kanara, but between 1942 and 1948 larvae were collected in North Kanara (Viswanathan, 1950).

Ecology: *A. culiciformis* has been collected in nature mostly as larvae from tree holes in extremely small numbers. Two adults were collected biting man and two more resting outdoors, in Pune (Ramachandra Rao and Rajagopalan, 1957). Cogill also made a collection from a ground pool. It is restricted to the heavy rainfall areas of the Western Ghats. No information is available regarding the adult resting or biting habits.

Relation to disease: No information.

Anopheles sintoni Puri, 1920

Type locality: Calicut (Kerala State), India.

Type: British Museum of Natural History, London, and National Institute of Communicable Diseases, Delhi.

Taxonomy: See under *A. culiciformis.*

Distinguishing characters: Adult is a moderate sized mosquito with a *culex* like posture. It differs from *A. culiciformis* by male genitalia and larval characters. For distinctions see Puri (1931), Christophers (1933) and Harrison and Scanlon (1975).

Distribution: Only known in India. First found in the west coast of Kerala. Neither the Bomaby Workers (Viswanathan, 1950) collected any specimen in North Kanara District during a six years' study nor Brooke Worth (1953) in Hassan District. Covell and Harbhagawan (1939) also did not find any specimen in Wynaad (Kerala).

Ecology/Bionomics: Larvae reported to have been found in tree holes by Puri.

Relation to disease: No information.

Anopheles barianensis James, 1911

Type locality: Barian, Near Murree, Pakistan.

Type: British Museum of Natural History, London.

Taxonomy: For sometime known as a variety of *A. plumbeus,* but Puri's study of larval characters indicated it to be a distinct species.

Distinguishing characters: Wings and palps completely dark; hind femur with distinct white spots at distal end (knee spots). Anopheline attitude.

Distribution: India, Pakistan, Tadzhik (U.S.S.R.).

In India: known only from the northern parts of Punjab, Himachal Pradesh (Simla, Kasauli, etc.) and Kashmir (Dal lake).

Ecology: A species which occurs at high altitudes (5,000-8,000 ft.). Adults have been collected biting man at dusk. Though it may enter houses, it chiefly rests during daytime out of doors, in holes of trees etc. It is quite a common species in Simla according to Christophers (1933). Larvae breed mainly in tree-holes. As with many other tree-hole breeding species it is perhaps cannibalistic. The larvae readily feed on crushed insects provided during artificial breeding which has been successfully done. [Refer Christophers (1933) and Puri (1931)].

Relation to disease: Unknown. The species generally occurs in areas with no or low grade malaria.

Anopheles lindesayi Giles, 1900 and subspecies nilgiricus Christophers, 1924

Type locality: Bakloh, Punjab Hill States (now in Himachal Pradesh).
Type: British Museum of Natural History, London.
Taxonomy: Christophers (1933) recognized five varieties which have been given subspecific status by Knight and Stone (1977). They are:

ssp *japonicus* Yamada, 1918, Japan, China, Korea;
ssp *nilgiricus* Christophers, 1924, India;
ssp *pleccau* Koidzumi, 1924, Taiwan, China;
ssp *cameronensia* Edwards, 1929, Malaysia; and
ssp *benguetensis* King, 1929, Philippines.

Only *A. l. nilgiricus* occurs in India in addition to the type form. The type locality for this subspecies is Nilgiri Hills, Tamil Nadu. Christophers had called it a variety but Puri (1949) raised it to subspecies status.

Distinguishing characters: *Adults*—Large dark mosquito, sometimes larger than *A. gigas*. Tip of hind tarsi not white; costa with less than 4 dark spots involving both costa and vein 1; hind femur without an outstanding tuft of black and white scales at its distal end as in *annandalei* and with a broad white band. There is much variation in the pale spots on the wings.

All terminations of veins dark, except 2.1 and 6 ..... *nilgiricus*
At least one other vein termination with pale spot ..... typeform

The subspecies *nilgiricus* further differs from the type form by: More golden as against silvery tint in the type form; hind femur dark at base except for a conspicuous milky white ring at extreme base about as long as femur is wide, and other characters.

Distribution: Type form: India, Pakistan, Nepal.

In India: Assam, Manipur, West Bengal, Kashmir, Himachal Pradesh, Uttar Pradesh, Punjab, Sikkim, all from the sub Himalayan regions. In a recent survey Ramachandra Rao *et al.* (1973) found the species in many districts (16 out of 28 visited) in Kashmir, Himachal Pradesh, Uttar Pradesh, West Bengal and Sikkim at altitudes ranging from 850 metres to 2700 metres. (see also Bhat, 1975a and b).

The subspecies *nilgiricus* is reported only from South India. It has been recorded at altitudes upto 8000 feet.

Ecology: It is a species occurring at high altitudes. Though it is a fairly common species and is wild in habits, specimens have been collected in houses entering for feeding (Strickland and Choudhury, 1927). Shortt (according to Christophers) recorded adults feeding throughout daytime in a small wood near Shillong. It has also been experimentally fed on man. It has been found resting outdoors. Bhat (1975a and b) collected engorged females from chicken coops and human dwellings and also while biting man.

In Nilgiris, Russell and Jacob (1942) recorded the species found as larvae at

altitudes of 1,200 metres. They were found usually in hill streams and once in a borrow pit. Larval breeding has been found in ground pools as also in rocky pools in mountain streams. Bhat (*loc. cit.*) found the species breeding in stream bed pools, paddy fields, rock holes, seepage pools and cement tanks usually in clean water in shade. The stream bed pools were the common breeding sites.

Relation to disease: There is one record of gut infection. No relation to malaria transmission is probable. Khan (1942) has also dissected nine specimens in Darjeeling with negative results.

Anopheles gigas Giles, 1901

Type locality: Coonoor, Nilgiris (Tamil Nadu), India.

Type: British Museum of Natural History, London.

Taxonomy: Christophers (1933) recognised four varieties viz., *formosus* Ludlow, 1901; *simlensis* James, 1911; *refutans* Alcock, 1913 and *baileyi* Edwards, 1929, of which only *simlensis* and *baileyi* occur in India. Variety *refutans* occurs in Sri Lanka. Var. *formosus* occurs in the Philippines. Knight and Stone (1977), however, recognised four more varieties and one subspecies, *viz.*, varieties *sumatrana* Swell and Roden; *danaubento* Mochter and Walandouw; *oedjalikalah* Nainggolen; *pantjarbatu* Naesoem Owinangoem and subspecies *crockeri* Colless, none of which occurs in India. These all forms are found in Indonesia and Borneo.

Distinguishing characters: Adults fairly large sized, among the largest of Indian anophelines, with markedly spotted wings, with less than three dark areas on costa involving both costa and vein-1. Inner quarter of costa mainly pale; hind femur without a broad white band. The type *gigas* and varieties in India can be distinguished by the following key.

Middle femur with a large pale spot at apical end on the dorsal side; no pale spots on outer half of vein 6 2

Middle femur without a large pale spot as above; vein 6 with a pale spot on outer half .... *gigas* type;

2 continuous dark fringe at vein 3 and onwards except for a pale area between veins 5-2 & 6 .... var. *baileyi.*

Fringe with a pale spot on vein 3 and at other veins following it .... var. *simlensis*

Distribution: India, Nepal, Bangladesh, Sri Lanka, Burma, and Sulawesi. In Burma, it has a very limited distribution in some of the areas on the Indo-Burma frontier. Perhaps occurs in Sumatra and Borneo. Stray records in Malaysia and none in Thailand.

In India, *A. gigas* is found at high altitudes generally 2,000 metres and above in the Nilgiris, Kodaikanal and Palani Hills, all in Tamil Nadu. Adult specimens have also been reared from larvae by the present author in North Kanara District at elevations of about 700 metres. In personal communication Mr. M.L. Shrestha

(1979) informs that both the type and variety *simlensis* have been found in Nepal.

All records from Burma are from larval collections (Khin-Maung-Kyi, 1971). They have been recorded at about 1,000 to 1,700 metres above sea level from forest streams during the months of March and April.

Bionomics/Ecology: Very little information exists on bionomics. It is comparatively a wild species occurring in forests or wooded areas. However, Christophers (1933) records that it is attracted to light and sometimes has been taken at Ootacamund (2,200 metres) inside buildings and that Cornwall succeeded in feeding it on pigeons but not on man.

The breeding places are however, better known. According to Puri (1931) the habitats are fresh water springs and ponds with vegetation at the edges, springs and seepages, ponds and small pools along the shallow hill streams. According to Cornwall (quoted by Christophers) it breeds in Coonoor (1,800 metres) all the year round.

Larvae have been well described by Puri and others. It is the largest anopheline larva in India, the length being 7 to 10 mm. Pupae are also described by Senevet.

The eggs described by Narayandas (1954), were compared with those of var. *refutans* showing that they were very similar in dimensions; *refutans* being marginally larger.

Relation to disease: No information, not likely to have any relation to human disease.

Anopheles gigas var. *simlensis* James, 1911

Type locality: Western Himalayas, Mahasu near Simla, and Murree, Pakistan.

Type: British Museum of Natural History, London.

Distinguishing characters: Adult is a large sized mosquito. It differs from the type form by apical portion of mid-femur with a large pale spot; without fringe spot on vein 6; inner accessory dark spot on costa short not extending to extreme base and shorter than the external spot; bands on palps more distinct; white scales on bases of femora whiter, broader and more conspicuous.

Distribution: Pakistan (Murree, Abbottabad), India, Nepal and Sri Lanka. (China?).

In India: Western Himalayas and Nilgiris, S. India. Christophers had given the distribution entirely in the Western Himalayas from Kashmir to Uttar Pradesh, at altitudes between 650 to 2,800 metres, the highest being at Gulmarg 8,500 ft. (approximately 2,440 mts.). In a recent survey, Ramachandra Rao *et al.* (1973) recorded this species in several localities in Kashmir, Himachal Pradesh and Uttar Pradesh. No specimens were found in West Bengal or Sikkim. Specimens were collected between 690 to 2,800 mts. Bhat (1975b) re-examining the material, further showed that the species was present at Kedarnath (3,530 mts.), probably the highest altitude at which it has been found. Varma and Mahadevan (1970) have found *A. gigas* in the foothills of the West Bengal area. As *A. gigas* type had hitherto been known only from South India, perhaps their collections were of *A. gigas simlensis*

or *baileyi,* which has been found in Sikkim by others. However, in view of Shrestha's statement mentioned above the question whether the type form occurs in Eastern Himalayas needs further study. Similarly, the record of *A. gigas* in Kashmir by Jacob (1950) may also be that of *A. gigas simlensis.* Puri (1948) recorded the species in Assam, Manipur as also from Kashmir, Kangra and Uttar Pradesh, but he does not say whether the form was one of the varieties or of the type. Russell and Jacob (1942) reported, its occurrence in the Nilgiri hills, Tamil Nadu. Pradhan, Shreshta and Vaidya (1970) however do not record it in their surveys of two areas in Nepal, but probably it occurs in that country. The variety normally occurs at higher altitudes though sometimes it is found in the plains just below the hills as for instance in Karnal, Haryana.

Bionomics/Ecology: The wild form does not enter houses (James and Liston, 1911). The females will suck human blood, Bhat (1975b) collected two engorged females from a hole in the ground made by a porcupine and two females while actually engorging on man.

It breeds in a variety of breeding places such as ponds, ground pools and ponds at the side of streams. According to Bhat (*loc. cit.*) the larvae were found in paddy fields, flooded wheat fields, shallow swamps, seepage pools, snow water pools, spring bed pools, forest pools, rock holes, rain water collections, and once in coal-tar barrels, with clean-water and various degrees of pollution and turbidity. Russell and Jacob (*loc. cit.*) found many larvae in hill streams in the Nilgiris that belong to both the typeform and variety *simlensis.*

Relation to disease: No information. Not likely to be a malaria vector because of its wild habits and also on epidemiological grounds.

Anopheles gigas var. baileyi Edwards, 1929

Type locality: Yatung, Tibet (at about 3,000 metres).

Type: British Museum of Natural History, London.

Taxonomy and Distinguishing characters: Very similar to var. *simlensis,* but distinguishable both as adults and larvae. (see key under *A. gigas*).

Distribution: India, Tibet, Burma, Taiwan, 'Indochina', Central China and Bangladesh.

In India: Eastern Himalayas, Assam and Meghalaya. Ramachandra Rao *et al.* (1973) and Bhat (1975a) have recorded this species in Sikkim, at altitudes of 1,950 to 2,750 metres. No specimens were collected in Western Himalayas. Specimens were all larvae collected from seepage pools.

Ecology: This species is prevalent at higher altitudes, but has also been found in the plains of Cachar District in Assam and Saidpur in Bangladesh. Though generally an outdoor rester, adults have been taken sometimes in cattlesheds and human dwellings in Shillong and upper Assam (Christophers, 1933). Larval breeding occurs in deep pools and in rocky pools or springs, and larvae have been collected practically in all seasons.

Relation to disease: Not known.

Anopheles annandalei Baini Prashad, 1918

Type locality: Sureil (altitude about 330 m), Darjeeling District, West Bengal.
Type: Zoological Survey of India, Calcutta.
Taxonomy: Two varieties, *A. djajasanensis* Burg, 1926 and *A. interruptus* Puri, 1929, had been recognized but *A. interruptus* has been raised to the status of a species by Reid (1968). Variety *djajasanensis* is found only in Java, Indonesia.
Distinguishing characters: A large tuft of dark and white scales, visible even to the naked eye, on distal end of hind femur is a characteristic of this group which includes *A. annandalei, A. interruptus, A. asiaticus* and *A. roniae* (the latter two not occurring in India). Another species, *A. wellingtonianus,* occurring in Malaysia only, also has a tuft of this kind but it is all dark, however it belongs to the *lindesayi* group and not to the *asiaticus* sub group.
Distribution: India, Sri Lanka and Indonesia (Java). In India: West Bengal (Darjeeling).
Ecology and relation to disease: *A. annandalei* is a species found at high altitudes. Little information is available on biology. It is less common than *A. interruptus.* Relation to disease unlikely.

Anopheles interruptus Puri, 1929

Type locality: Sukna, Darjeeling District, West Bengal.
Type: British Museum of Natural History, London; Zoological Survey of India, Calcutta.
Taxonomy: Puri (1929) first regarded this as a variety of *A. annandalei,* but later (1949) raised it to the status of a subspecies so listed by Stone *et al.* (1959). However, Reid (1968) has raised it to the status of a full species. Knight and Stone (1977) now list it as a full species.
Distinguishing characters: *Adult:* A small sized species distinguished by a conspicuous tuft of scales on the apical part of hind femur even detectable by naked eye; the tuft is white distally and dark proximally. It shares this character with *A. annandalei* (present in India) and *A. asiaticus* Leicester, 1903, (occurring in Thailand and Malaysia). The two Indian forms can be distinguished from each other as follows:

> Subcostal spot present; mesonotum more scaly; more numerous scales on pleurae; dorsal anterior pleural hairs of all segments with fine barbs .... *interruptus.*
> No subcostal pale spot; dorsal anterior pleural hairs simple ... *annandalei.*
> (adopted from Christophers, 1933).
> A closely related species is *A. asiaticus* of Malaysia and Thailand.

Distribution: India, Nepal, Sri Lanka, Burma, Thailand, Malaysia, Kampuchea, Vietnam, Yunnan Province in China. In India: Eastern Himalayas, Darjeeling District, Assam and Khasi and Jaintia hills (Meghalaya). Russell and Jacob (1942)

recorded it from Nilgiris, South India. It has been recorded from Coorg District, Karnataka, Rama Rao and Achuthan, 1964 Kumar, 1960 and in Shimoga and Hassan Districts (Rao *et al.*, 1952).

Bionomics/Ecology: A comparatively rare species which occurs in deep forest. Adults have been collected by Puri in outdoor places. It is also known to feed on man in Thailand between 19.00-20.00 hours. It was first recognised in Malaysia in collections on human bait in the forest.

The larvae are tree-hole breeders and have been collected upto the altitude of 900 m. Larvae are very dark and not easily seen in the dark waters of tree holes.

One adult and 28 larvae were collected in Coorg District. The larvae were found breeding in association with *Toxorhynchites* spp. All the 28 larvae were collected from one tree hole. No information is available on other aspects of biology.

Relation to disease: No information, not likely to be of any significance.

Anopheles argyropus (Swellengrebel), 1914

Type locality: Deli (Sumatra), Indonesia.

Type: The original type believed non-existent. However, Reid (1953) designated "plesiotypes" from Malaysia and placed them in the British Museum of Natural History, London.

Taxonomy: It was originally named *Myzorhynchus argyropus* by Swellengrebel in 1914 and later was regarded as an aberration of *A. hyrcanus* by Christophers (1933). Reid (1953) resurrected it to the status of a species.

Distinguishing characters: The species very closely resembles *A. nigerrimus* and *A. peditaeniatus*. Harrison and Scanlon (1975) felt that it is more closely related to the former than to the latter. It differs from *A. nigerrimus* in not having a pale band at the base of the third palpal segment of male; absence of any pale scales on the base of costa; having parallel vertical wrinkles on the pupal trumpet, and having a somewhat intermediate number of long pecten teeth (*nigerrimus* 4-7; *argyropus* 6-8; *peditaeniatus* 7-9).

Distribution: India, Burma, Thailand, Kampuchea, Indonesia, Malaysia and Vietnam. Knight and Stone (1977) do not include Kampuchea and Vietnam, but Harrison and Scanlon (1975) do so.

In India: in Assam region only. (The Indian records are based on studies by Reid and Harrison and Scanlon).

Bionomics/Ecology: Very little is known about the habits and habitats of this species in India. In Thailand and Malaysia it has been collected in small numbers in houses and cattle sheds and seems to be more attracted to cattle than to man. Light traps have provided more material than dwellings. Breeding places are rice fields and swamps.

Relationship to disease: There is no evidence to suggest any relationship of this species to human malaria or filariasis. It has also been found refractory to the monkey malaria parasite, *P. cynomolgi bastianellii* (Warren *et al.*, 1963).

Anopheles crawfordi Reid, 1953

Type locality: Kuala Lumpur, Malaysia.

Type: British Museum of Natural History, London.

Taxonomy: Till Reid clarified the taxonomy of the *hyrcanus* group of species, this species was probably being identified as *A. sinensis* because of the similarity in general appearance.

Distinguishing characters: Very similar to *sinensis*. Can be distinguished only with wing characters and pleural chaetotaxy; but no characters are absolute. Larval identification is also difficult and is based on the branches on abdominal seta-5 on segment-2. Entomologists working in the eastern region of India should be on the lookout for this species. For details, use the key given under *nigerrimus* and also refer to Reid (1968). The adults can be distinguished by a combination of the following characters:

Bands on hind tarsi narrow; wide apical pale wing fringe spot, coxae and male basimeres with pale scales; wings with dark spots sharply defined and tip of vein-1 pale; no basal pale band on third segment of male.

Distribution: India, Thailand, Malaysia (Peninsular), south Vietnam, Kampuchea and Indonesia (Sumatra).

In India from "Assam" only.

Harrison and Scanlon (1975) state that they have seen specimens from India in the collections in the U.S. National Museum.

Bionomics/Ecology: Nothing is known about the ecology of *A. crawfordi* in India. However, in Thailand and Malaysia where it is more common it seems to be associated with heavy rain and wet forests. The larvae occur in several types of habitats, particularly marshes and ground pools. Adults have been collected in man-baited traps and also biting man and buffaloes. The species is more attracted to man than monkeys (Wharton *et al.*, 1964). *A. crawfordi* seems to bite man in numbers in certain localities (Harinasuta *et al.*, 1964).

Relation to disease: There is no record of relationship to malaria. Though experimentally infected with *Brugia malayi*, it is not regarded as an important vector of filariasis because of its zoophily and scarcity (Reid, 1962 and Wharton *et al.*, 1963).

Anopheles nigerrimus Giles, 1900

Type locality: Calcutta, (India).

Type: British Museum of Natural History, London.

According to Harrison and Scanlon the specimen, a holotype female, is in poor condition and some parts such as palps, antennae, etc. are missing.

Taxonomy: This species has long been known in India as a variety of *A. hyrcanus* (Pallas), 1771. Christophers (1924 and 1933) reviewed the synonomy of *A. hyrcanus* Pallas and its varieties. His view was that all these forms were difficult to distinguish in individual adults and the close similarity in the leaflets of the phallosomes and certain larval characters seemed to indicate that they were no more than varieties.

A. hyrcanus sensu Pallas is not regarded to be present in the Indian subcontinent.

Extensive taxonomic work on the *Anopheles hyrcanus* group in Southeast Asia has been done in Malaysia by Reid (1953 and 1968), and in Philippines by Baisas and Hu (1936) and also in Japan and China. Harrison and Scanlon (1975) state that the material examined from Thailand agreed very well with Reid's observations in Malaysia. Based on Reid's classical work the following species of the Southeast Asian *hyrcanus* group have now been recognized:

> *A. argyropus, A. nigerrimus, A. nitidus, A. pursati, A. pseudosinensis* (in the Philippines only) *A. sinensis, A. lesteri lesteri, A. lesteri paraliae, A. crawfordi,* and *A. peditaeniatus.* Four other forms occur in China, Korea and Japan.

Of these *A. argyropus, A. nigerrimus, A. nitidus, A. sinensis, A. peditaeniatus* and *A. crawfordi* are recorded from India. Harrison (1972) recognized two subgroups (i) the *nigerrimus* subgroup to include *nitidus, nigerrimus, pursati* and *pseudosinensis* and (ii) the *lesteri* subgroup including *lesteri lesteri, lesteri paraliae, crawfordi* and *peditaeniatus.* The two remaining species *argyropus* and *sinensis* are isolated. Harrison slightly differed from the interpretation of Reid (1968). All these indicate the extremely complex nature of the assemblage of species of this group.

The true *A. hyrcanus* Pallas, 1771 has not been recorded in India. According to Knight and Stone (1977) the distribution of the true *A. hyrcanus* occurs in Central and Northern Asia, Northern Mediterranean, Libya, Japan and Hungary. It is worth noting that *pseudopictus,* a synonym of *A. hyrcanus* occurs in North Afghanistan.

Christophers (1916) had first named the form now called *nigerrimus* as var. *vanus* but the name *vanus* had already been used for a species in the *barbirostris* group. Therefore, the name *nigerrimus* had priority and was adopted.

Distinguishing characters: In view of the extensive work on the taxonomy of the members of *A. hyrcanus* group, it is desirable to provide a key for all of them. The differences between the members of the group are shown in tabular form (Table, 22a).

A. nigerrimus is difficult to distinguish from its close relatives owing to the extreme variability shown by the members of this group and the possibility of misidentification exists. But in most parts of India where neither *A. peditaeniatus* nor *A. sinensis* occurs the species is not difficult to recognize. It is only in Assam and neighbouring areas that workers have to be very watchful. It can be distinguished from all its relatives by a combination of the following characters:

Inner quarter of costa mainly dark though some scattered pale scales may occur; palpi with distinct pale markings; long dark mark on base of wing vein 5; fringe spot on vein 5.2.

Distribution: Throughout the Oriental region from south-east Afghanistan to Indonesia. It does not occur in the Philippines, the group being represented there

Table 22a. Some distinguishing characters of members of the hyrcanus group in India

	nigerrimus	nitidus	peditaeniatus	crawfordi	sinensis	argyropus
Adult						
Hind tarsal bands	Narrow, extending to basal part of the joints very slightly as in joint 4.	Broad extending across joints.	Broad extending to both sides of joint, wider.	Narrow apically.	Narrow apically.	Very broad (some geographical variations).
Palp:						
Female	4 clear bands, infrequently apical and pre-apical bands confluent.	4 bands always present, the two basal bands narrow.	4 bands present but rarely apical and pre-apical bands confluent.	4 clear bands, sometimes apical and pre-apical bands confluent.	4 clear bands.	As in *peditaeniatus*.
Male	A pale ring on basal third of stem.	A pale band on basal third of stem.	No basal pale band on Segment III.	No pale band on base of Segment III.	Middle of stem without a pale band.	No pale band on base of Segment III.
Wing: Costa	Some pale scales on basal third costa.	Scattered pale scales on the basal third of costa.	Basal third of costa completely dark.	Basal third of costa entirely dark.	Basal half of costa entirely dark, only sub-costal & pre-apical pale spots present.	No pale scales on basal third of costa or few only.
Scales on humeral cross vein	Present.	Present.	None or 1-2.	None or 1-2.	Present.	Present.
Fringe spots on 5.2.	Present.	Present. (often)	Absent.	Absent.	Usually present.	Absent.
Basal mark on vein 5.	Long.	Short.	Long.	Short.	Short.	Long.
Larva:	Many branches on the sutural hair; greenish brown or black with transverse bands.	Commonly green with no transverse bands.	Usually dark brown, without transverse pale bands.	Greenish colour with transverse bands.	Greenish or brown, with faint transverse bands.	Usually dark brown without transverse pale bands.
Eggs	Undivided narrow deck.	Divided deck.	Undivided broad deck.		Broad deck.	Very narrow deck.

by *lesteri, peditaeniatus* or *pseudosinensis.*

Christophers (1933) records that Puri had examined larval skins from West Asia, indistinguishable from *A. nigerrimus.*

In India, it occurs in all parts of the mainland of India. It is scarce in the higher elevations in the Himalayas, but has been recorded from Kathmandu, Nepal (Puri, 1948). It is not reported from the Andamans or Lakshadweep Islands.

Prevalence

A. nigerrimus is normally a species of lower altitudes occurring in all zones of India, but occasionally has also been found occurring at elevation of 1,800 m. above sea level in parts of eastern India and in the Nilgiris. It is likely to be found also in localities far removed from houses or cattle sheds.

Adult Bionomics

Resting habits: Adults are generally rare inside dwellings during daytime, though a few specimens can be found when intensive and extensive searches are made. Covell (1944) recorded them in cattle sheds and Russell and Ramachandra Rao (1940a) in small numbers in human and animal dwellings in South India. Senior White and colleagues recorded a small number inside dwellings in east central India but Covell and Pritam Singh (1942) found that *A. nigerrimus* was a rare species in the Chilka lake area of Orissa, unlike in many parts of India. Though a fair amount of breeding of the species was going on in the vicinity of Pune, Viswanathan *et al.* (1955), did not find the species entering experimental huts in which all night collections were made indicating a reluctance to enter man-made shelters.

However, Russell *et al.* (1938) made some significant observation in this regard. Adults of *A. nigerrimus* were sometimes found in houses and cattle sheds, but the numbers did not adequately represent the true prevalence of the species. In Pattukkottai area they found only 123 adults during a two year period when over 121,283 adults of all the 12 anopheline species occurring in the area were captured. However, in a calf baited magoon type of portable trap in which there was a 'V' shaped entrance, which while giving free access to the mosquitoes prevented their exit, 5,339 females of *A. nigerrimus* were collected in 24 months in 350 collections. For a comparison, the numbers of the different species collected in houses and cattle sheds and in the magoon trap are given below:

	Houses and cattle sheds (man hours) Two years	A single magoon trap baited with a calf (350 collections)
A. aconitus	25	Nil
A. annularis	2,064	93
A. barbirostris	8	33
A. culicifacies	15,942	525

A. nigerrimus	123	5,339
A. jamesii	544	23
A. pallidus	214	256
A. stephensi	5	Nil
A. subpictus (females)	46,468	17,181
A. tessellatus	83	8
A. vagus (females)	14,904	1,477
A. vagus or *subpictus* (males)	14,130	118
A. varuna	773	56
	121,283	25,109

It can be observed that the house resting behaviour of *A. nigerrimus* was exactly the opposite to that of *A. culicifacies*. This study gave an indirect indication of the degree of outdoor resting of the different species. It also showed that *A. nigerrimus* is not altogether averse to enter a man-made structure, perhaps left it for outdoor shelters immediately after feeding.

Feeding and biting habits: No detailed studies have been made of the feeding habits of this species in India, but it is generally recognized that *A. nigerrimus* feeds predominantly on cattle. Presumably it feeds on wild animals also as it is sometimes found far from human or animal shelters. However, it bites man readily. The present author has several times experienced massive attacks by females of *A. nigerrimus* at dusk in Pattukkottai when he was out in the evenings looking for swarming and mating of mosquitoes in the open in proximity of rice fields and quite away from habitations. Numerous, sometimes as many as 20 or 30, hungry adults have attacked him within a period of about 15 minutes just after sunset during failing light. Similar observations though not of the same intensity were also made by him in North Kanara. Ramachandra Rao and Rajagopalan (1955) collected a few specimens biting on human volunteers in Pune. Bruce-Chwatt *et al.* (1966) in their review of the host selection by anopheline mosquitoes pointed out that *A. nigerrimus* was primarily zoophilic. Though the adults, are reluctant to stay indoors during daytime, specimens have sometimes been actually collected biting man and cattle inside dwellings. Moorehouse (1965) found adults biting man outside as well as inside houses in Malaysia.

Very little information exists on flight, dispersal and other biological activities.

Larval Ecology

A. nigerrimus has been known to breed in various types of still waters with aquatic vegetation and it has been particularly associated with rice fields. In Thanjavur District in Tamil Nadu, *A. nigerrimus* was predominantly a rice fields breeder. It was also commonly found in field channels closely associated with rice fields. Out of 7,232 larvae of all species collected on 742 occasions in growing rice fields, 6,138 *A. nigerrimus* larvae were collected on 499 occasions (Russell and Ramachandra

Rao, 1940). When the succession of species in rice fields was studied in greater detail, it was found that *A. culicifacies* and *A. subpictus* were the predominant species in fallow or freshly ploughed fields, *A. pallidus* in fields with freshly planted rice and middle stages of growth and *A. nigerrimus* appeared in fields with fully grown plants. It cannot be stated whether it is due solely to the presence of rice plants or to the increases in algal growths or both. Similar observations have been made in Bengal (Sen, 1948b).

Relation to Disease

In thousands of dissections made by many workers in India, *A. nigerrimus* has not been found naturally infected (Wattal, 1961b, Horsfall, 1972). This is extremely interesting because the closely related *A. sinensis* has been found infected with both gut and gland infections in many areas in the Oriental region. Even as regards *A. sinensis* doubts are now being expressed because of the recent taxonomical clarification. Probably it was *A. lesteri* which was the vector (see under *A. sinensis*). Some earlier reports by Hodgkin (1956) of *A. nigerrimus* being a vector in Malaysia have not been substantiated as there is some doubt because of the possible confusion of identification among members of the group. Harinasuta *et al.* (1976) include *A. nigerrimus* as a malaria vector in Indonesia.

A. nigerrimus has long been suspected to be a vector of human filariasis and natural infections have been found in West Bengal, Thailand and Malaysia. In a series of experiments made in Sri Lanka, Giles (1961) made the following observations:

A. nigerrimus can easily adapt itself to laboratory maintenance. The adults mate readily in cages 3 × 3 × 3 feet and feed readily on man, cats, dogs, guinea pigs, rabbits and fowls. It was experimentally infected with *W. bancrofti, B. malayi* and *S. digitatus* as the following data would show:

	No. fed.	No. with developing forms.	No. with infective forms.	Total No. of infective forms in surviving mosquitoes.
W. bancrofti	25	14	9	38
B. malayi	25	9	15	64
S. digitatus	25	10	15	78

Raghavan (1969), in his review of the vectors of filariasis, had shown that there were records of natural infections of *B. malayi* in *A. nigerrimus* (1.18 per cent) in Calcutta (Ghosh and Hati, 1966) and of experimental infections (85.5 per cent) with *W. bancrofti* (S.S. Rao and Iyengar, 1932). Roy and Sharma (1960) found three specimens of *A. nigerrimus* infected with *W. bancrofti* in Cuttak District. There are several reports of *A. nigerrimus* being a vector of *B. malayi* in Thailand, Borneo, Sri Lanka, etc., but they could as well have been infections of *Setaria* spp. Iyengar

(1953) recorded positive natural infections while studying *B. malayi* in Thailand, but doubts have been raised whether the infections could not have been due to animal filaria (Reid, 1968). Reid *et al.* (1962) had shown that *A. nigerrimus* could be infected with *B. malayi,* but in a very low degree.

The probable role played by some anophelines of transmission of virus diseases has been discussed elsewhere. It is interesting to note that *A. nigerrimus* has been naturally found infected with Arkonam virus in Tamil Nadu (Dandavate *et al.,* 1969) and with Japanese encephalitis virus in West Bengal by Chakravarty *et al.* (1975) and by the National Institute of Virology, Pune (1979).

Anopheles nitidus, Harrison, Scanlon and Reid, 1973

Type locality: Selangor, Malaysia.

Type: Holotype, female in British Museum deposited by Reid as *A. indiensis.* Allotype, male, also in British Museum of Natural History; Paratypes in U.S. National Museum.

Taxonomy and distinguishing characters: Theobald (1901) first described *indiensis* as a subspecies of *A. sinensis* and the type female was deposited in British Museum. Christophers (1924 and 1933) synonymized it under *A. nigerrimus.* Harrison and Scanlon (1975) say 'This species was previously called *indiensis* until Harrison, Scanlon and Reid (1973) determined that *indiensis sensu* Reid (1953, 1968) does not occur in Madras, the type locality of *indiensis* Theobald. Since the type of *indiensis* Theobald, 1901, is believed lost or non-existent and no other specimens are known, *indiensis* of Theobald was synonymized under *nigerrimus* Giles, 1900, and *indiensis* of Reid was renamed *nitidus.* Therefore, the name *indiensis* of Theobald is no longer extent and *indiensis* of Reid is now only a synonym of *A. nitidus.*

Christophers (1924, 1933) had placed *indiensis* of Theobald as a synonym of *A. nigerrimus.* During their extensive collections of anophelines in Madras and neighbouring areas Russell and co-workers including the present author had not recorded specimens of *indiensis* but it must be admitted that the characters which distinguish *indiensis* from *nigerrimus* are so slight that they may have been missed.

Distinguishing characters: The adults very closely resemble *A. nigerrimus* and *A. sinensis* but are recognised by a few characters, *viz.,* wide pale bands on the hind tarsi; wing pattern brighter; pale scales on the basal third of the costa and on preapical dark mark on vein 1; and a patch of dark scales on the humeral cross vein 1. Males with a pale humeral spot on costa and a basal pale band on palpal segment-3; mesonotum usually with "eye-spots". Larvae and pupae difficult to distinguish (refer for details to Harrison and Scanlon, 1975).

Distribution: India, Thailand, Indonesia (Sumatra), Malaysia (including Sabah) Kampuchea, South and North Vietnam.

In India in Assam only (specimens actually seen by Harrison and Scanlon).

Ecology: Nothing is known about the habits and habitats of *A. nitidus* in India. The observations in this regard have been made in Thailand and Malaysia. It is a foot-hill species. It has been found to bite man and in some localities may even be

the largest component of the "*hyrcanus* group" (Scanlon and Sandhinand, 1965). However, it is largely a cattle feeder. The breeding places are swamps, marshes in jungle, seepages, large mine-pits, rock pools, ditches, rice fields, and elephant foot-prints in the forest.

Relation to disease: No information is available regarding its relationship to disease in India. All that we know is from observations made in Thailand and Malaysia. There is no evidence of its being involved in human malaria transmission. It has a low susceptibility to the monkey parasite, *Plasmodium cynomolgi bastianelli*. A few adults are attracted to monkey baited traps. The species has also not been found with human filaria infections but Reid *et al.* (1962) found this to be capable of being infected experimentally by *Brugia malayi*.

Anopheles peditaeniatus Leicester, 1908

Type locality: Kuala Lumpur, Malaysia.

Type: Syntypes selected by James and Stanton in 1912 in British Museum. The original types by Leicester have been regarded as lost.

Taxonomy: Originally described by Leicester as a species of *Myzorhynchus,* but subsequently placed under subgenus *Anopheles.*

Distinguishing characters: *Adult*—A large sized mosquito with a superficial resemblance to *A. nigerrimus.* Distinguished mainly on the basis of the wing characters and the width of hind tarsal bands which extend across joints. But in India the hind tarsal bands may not show the difference. However, this species can be distinguished from *A. nigerrimus* by having a line of white (not dark) scales along the anterior border of the remigium; humeral cross vein bare of scales; no pale scales on basal half of costa and no fringe spot on vein 5.2.

Larva: Mesothoracic hair No. 4 small with sinuous branches arising close together near the base.

Distribution: India, Nepal, Bangladesh, Burma, Thailand, Kalimantan, Malaysia, Vietnam, Sulawesi, Philippines, China (Fukien, Kwechow, Yennan provinces) and Sri Lanka.

In India: recorded from Assam, West Bengal, Bihar, Madhya Pradesh, Tamil Nadu, Karnataka and Punjab.

According to Harrison and Scanlon (1975) though the species occurs in small numbers, probably it has the widest distribution among all Southeast Asian members of the group even exceeding that of *A. sinensis* which has hither to been regarded as the widest distributed species. Probably many specimens have been missed by entomologists working in India because of its resemblance to *A. nigerrimus.*

Prevalence/bionomics/ecology: Very little information exists on the biology of this species in India. Reuben (1971) during her studies on the mosquitoes of North Arcot District, Tamil Nadu, collected quite a good number of adults inside houses as well as in cattle baited traps. Observations made in Thailand, indicate that the adults have been collected biting man both inside and outside houses and also

biting cattle. It has also been collected in many kinds of traps. Cattle sheds appear to be preferred to houses for resting. Cattle are the overwhelmingly preferred hosts.

The breeding places are mainly rice fields, but the larvae have been collected from marshes, ditches, seepages, ponds, swamps, temporary pools, margins of streams, animal foot prints, shallow well etc. indicating a wide breeding habit.

Adults have also been collected at altitudes of 570 m. in Sri Lanka and 540 m. in Thailand. The present author has seen specimens collected in Mysore District made by the National Institute of Virology, at altitudes about 600 m. The species occurs in North Arcot District of Tamil Nadu at less than 30 m.

Relation to disease: The species is not known to have any relationship to human malaria. Experimentally it has been found to have a very low susceptibility to *P. cynomolgi bastianellii*. It is also a good experimental vector of *B. malayi*, but there is no evidence of its taking part in natural transmission of human filariasis. Perhaps it is a vector of the animal filaria genus *Setaria*.

Anopheles sinensis Wiedemann, 1828

Type locality: Canton, China.

Type: Natural History Museum, Vienna, Austria. Lectotypes and paralectotypes have been deposited by Harrison. The two lectotypes in the Vienna Museum belong to the original series of Wiedemann and are stated to be in poor condition. Presumably the holotype of Wiedemann is lost. A female lectotype and a female paralectotype also seem to have been designated by Harrison in University Zoological Museum, Copenhagen, Denmark. The type specimens of a synonym, *A. plumiger* Donitz, 1901, are stated to be in the Zoological Museum, Humboldt University, Berlin.

Taxonomy: Christophers had originally regarded this species as a variety of *A. hyrcanus* Pallas, but it has now been given the status of a species (Reid, 1953, 1968; Knight and Stone 1977; Harrison and Scanlon, 1975).

Distinguishing characters: Because of extreme variability of the characters used for identifying adults, pupae and larvae of the members of the *hyrcanus* group, the identification of individual specimens of *A. sinensis* is difficult. Variations occur in size, wing markings, larval chaetotaxy and even in the selection of breeding places. From *A. nigerrimus*, the most common species, in India, *A. sinensis* can be distinguished by the following characters:

Basal dark area on vein-5 short equal to or less than basal pale mark on same wing; basal third of costa entirely dark without pale scales.

For distinguishing *A. sinensis* adults from other members of the group, see under *A. nigerrimus*.

Distribution: Very extensively prevalent in the Oriental region, from India through Nepal, Burma, Thailand, Malaysia (Peninsular), Indonesia (Sumatra), China, Kampuchea, Vietnam, Manchuria, Taiwan, Japan. The present author had seen specimens in North Afghanistan in 1950.

In India, it is an extremely scarce species, so far found only in 'Assam' region

(Manipur). Some dissections of this species have been recorded by Subramanian and Sen Gupta (1950) in Madhya Pradesh with negative results but doubt exists as to the accuracy of the identification.

Prevalence

Practically nothing is known about the biology of this species in India because of its very restricted distribution. Practically all the information on biology has originated from the countries. In Burma, where also it has a limited distribution, it occurs in large numbers in a few areas. For example, at Myitkyina, near the Indian border Watson (1944) (quoted by Khin-Maung-Kyi) recorded a large number of adults in night catches.

The species is mainly associated with agricultural areas rather than with deep jungle or forests.

Adult Bionomics

Resting habits: In the entire range of its distribution adults are found resting in human and animal dwellings, but it is regarded as generally exophilic.

Biting habits: In Burma, Watson (1944) (quoted by Khin-Maung-Kyi) collected 117 specimens out of 278 of all species entering tents at night but it is stated (Fox 1949, quoted by Khin-Maung-Kyi) that as cattle were not present during Watson's investigations the observation had a limited value. Khin-Maung-Kyi reports adults being caught on cattle before midnight but not a single specimen on human baits. In Thailand also no *A. sinensis* adults were collected during human biting collections in an elevated semi-forested area though 9,303 mosquitoes of all types were collected. Scanlon and Esah (1965) in Chiangmai Valley collected adults biting man at an altitude of about 300 metres but not at higher elevations. Wilkinson *et al.* (1970) also recorded only one adult in 15 night collections in a jungle camp. In Malaysia, Reid (1968) also records similar observations. When dry ice was used to attract mosquitoes moderate numbers were collected. Ample observations made in other areas such as Vietnam and China have shown that the species is predominantly zoophilic. Though the species is obviously highly zoophilic it does bite man also as seen from the results of precipitin tests where high percentages of blood from mosquito stomach have shown reaction to anti-human sera (See Horsfall, 1972).

Longevity: From laboratory observations the life span seems to be a maximum of 57 days (Kingsbury, 1935) (quoted from Horsfall, 1972) and according to Treillard (1933) (quoted from Horsfall, 1972) less than 10 per cent live longer than three weeks.

Oviposition: Chow (1948) (quoted from Horsfall, 1971) showed that the gonotrophic cycle was 2 to 4 days and the number of eggs laid per batch varied from 115 to 255.

Larval Ecology

In Southeast Asia [Strickland, (1936), Harrison and Scanlon (1975) and Reid (1968)] *A. sinensis* breeds in a variety of habitats of which rice fields seem to be the most important of them. Other breeding places such as marshes, streams, seepages and various types of ground pools are also common. Harrison and Scanlon state that this species has not been found in brackish waters and also that Blakeslee notes that the species does not breed when salinity is more than about 6 per cent of sea water. Studies in China have shown that it has similar breeding habits [Chang (1940) and Yao & Wu (1934), quoted from Horsfall (1972)]. The species there breeds essentially in still waters but some breeding takes place in flowing waters also. Senior White (1928) dissected some larvae and found the species to feed on all types of plankton and suspended matters. Larvae are predominant in Thailand between October and November when the rice fields are fully flooded and in Burma at the end of the monsoon months of August and September (Khin-Maung-Kyi 1971). As in the case of *A. nigerrimus,* larvae appear to be more predominant during the later phases of rice cultivation than in the earlier ones.

According to Horsfall (1972) "The Indian population is very large and found in all sorts of places, especially abundant in ponds with water hyacinth, swamps and borrowpits". No recent records are available.

It should, however, be noted that some of the earlier observations on the species may have to be reviewed in the light of the taxonomical changes brought about by the work of Reid.

Relation to Disease

A. sinensis was regarded till recently as an important vector of malaria, particularly in China. Many gut and gland infections have been recorded, but considerable doubt is now being expressed as to its true role in malaria transmission. Numerous gut infections have been found in Burma, Taiwan and Indonesia by several workers. Infected specimens have also been found in "Indochina", Indonesia (Sumatra, Java, and Kalimantan). However, not only the specific identification of the species is in doubt, but also the nature of the plasmodia, whether human or animal. Ho *et al.* (1962) considered both *A. sinensis* and *A. lesteri* as the vectors of malaria in China, but Reid, in reviewing their paper (*Tropical Diseases Bulletin* July, 1962) commented that the probable vector of malaria in Central China is not *A. sinensis* as has been long believed but the related species *A. lesteri.* The name *sinensis* has covered a number of species of different habits but similar in appearance. At any rate, the role played by *A. sinensis* as a malaria vector in Southeast Asia seems to be minimal.

Wattal (1961) gives no record of dissections in India, except for the doubtful record by Subramanian and Sen Gupta (1950) referred to above, no other records are available. The role of this species in India is doubtful.

The role of *A. sinensis* as a vector of human filariasis is also doubtful. The posi-

tive dissection results of Iyengar (1953) in Thailand with infections of *B. malayi* are now questioned as the identification of the species was made before the revision of the group by Reid (1953). Reports of natural infections are also now regarded as due to infections by animal filaria parasites, but in China the species is regarded as an important vector of human filariasis.

Anopheles ahomi Chowdhury, 1929

Type locality: Upper Assam, India.

Type: Location unknown.

Taxonomy: Christophers (1933) regarded it as a vector of *A. barbirostris* but Reid (1962) raised it to the status of a species.

Distinguishing characters: From *A. barbirostris* it is distinguished by absence of white scales on the venter of the abdomen; inner clypeal with fine lateral branches in the apical portion.

In *A. barbirostris* the venter has white scales (except perhaps in specimens from the Andamans); larvae have inner clypeal simple or rarely bifurcated.

Distribution: India and Burma.

In India known from "Assam" area only.

Bionomics/Ecology: Little information.

Relation to disease: Unknown, and unlikely.

Anopheles barbirostris Van der Wulp, 1884

Type locality: Mount Ardjoeno, Java, Indonesia.

Type: State Museum of Natural History, Leyden, Netherlands.

Taxonomy: Reid (1962, 1968) lists 11 related species, divided into two subgroups:

(a) Barbirostris subgroup:

A. barbirostris	Pakistan to Indonesia and South China.
A. campestris	Closely related to above and common in coastal regions of Malaysia. (East Coast).
A. donaldi	Principal member of the group in Borneo.
A. franciscoi	The Philippines.
A. hodgkini	Widespread in Thailand, Indonesia, Malaysia & Burma. (may occur in the Andamans).
A. pollicaris	An uncommon but easily recognizable species in the forests of Malaysia and Thailand.

(b) Vanus subgroup:

A. ahomi	In 'Assam', India.

A. vanus	Sulawesi, Moluccas, Kalimantan and the Philippines. Doubtful record in India.
A. barbumbrosus	Replaces *vanus* in the Western Archipelagos and Moluccas. Recorded from India.
A. manalangi	In the Philippines.
A. reidi	An unusual species in Sri Lanka which had been previously recognised as *A. pseudobarbirostris* Ludlow, which is a member of the *bancrofti* group and is found in Sulawesi, the Philippines (Mectan), Moluccas and New Guinea. This was recognised by Harrison (1973a) and the form renamed *A. reidi*.

Of these species, *A. barbirostris, A. ahomi* and *A. barbumbrosus* are reported from India.

A tabular key for distinguishing characters of the three species found in India and *A. campestris* is given in Table 22b.

Distinguishing characters: A comparatively large mosquito with a shaggy appearance. Till Reid (1962) studied this group and made extensive changes in taxonomy is Southeast Asia, *A. barbirostris* was comparatively easy to identify in India. Even now it can be distinguished without much difficulty from other members of the group except *A. campestris,* which is not recorded in India.

A. barbirostris females can be recognised by a combination of the following characters: Proboscis dark; palp shaggy and entirely dark; absence of an accessory pale fringe spot on wing vein 2.1; a narrow pale fringe spot confined to vein 3; another fringe spot on 5.2; a tuft of black scales on sternite 7; an abundance of pale scales on vein 5; a few pale scales on abdominal sterna; narrow apical pale bands on fore tarsi. Larvae can be identified by palmate hair on abdominal segment 2; stiff broomlike branches on outer clypeal at least 40 in number; spiracular apparatus does not possess a sigmal process. There is need for further close examination of specimens of *A. barbirostris* from different parts of India to determine whether *A. campestris* also occurs.

Wattal *et al.* (1962) after studying all the collections in the National Institute of Communicable Diseases, considered that *A. barbumbrosus* and *A. vanus* also occur in India, but Reid (1968) considered *A. vanus* of Wattal *et al.* as equal to *A. barbirostris.* It is essential for students of Indian anophelines to re-examine fresh material from this country to find out if any of the other members of the group are present. The key given by Harrison and Scanlon may be consulted.

Table 22 b. Some distinguishing characters of the barbirostris group in India

	barbirostris	ahomi	barbumbrosus	campestris*
Adult				
Wing:				
Fringe spot on 2.1	Absent	—	Many with fringe spot on 2.1	Absent.
Lower apical fringe spot.	Narrow opposite vein 3 only.	Atleast from vein 3 to 4.1.	Broad, atleast from veins 3 to 4.1.	Narrow opposite vein 3 only.
Stem on vein 5 between basal dark mark and fork.	Dark scales, less than half.	—	—	Dark scales more than half.
Abdomen:				
Ventral pale scales.	Venter with white scales, median, few on lateral margins.	No ventral white scales.	No ventral white scales.	Ventral white scales more than in *barbirostris*.
Larva:				
Outer clypeal hair.	With many (over 40), stiff branches and broomlike.	With thin branches 12-36, rarely more than 40, side branches present.	Branches fewer than in all others, thinner with no side branches.	Hairs more bushy than in *barbirostris*.
Inner clypeal hair.	Simple or rarely bifid.	With fine lateral branches on apical portion.	Not branched.†	As in *barbirostris*.
Egg:	Undivided deck.	—	—	Undivided deck.

*Does not occur in India, but an important vector in Malaysia.
A. campestris is very similar to *barbirostris* except that the ventral pale scales on abdomen are more.
†Christophers says: IC with fine lateral branches.

Notes:

1. *A. campestris* is included in Table 22b because of its close similarity to *A. barbirostris*. Though it has not been reported from India, it is suspected to occur by Reid and also by Harrison and Scanlon.
2. *A. reidi* which occurs in Sri Lanka differs from *A. barbirostris* by the following characters (Harrison, 1973): (1) spotted legs, (2) a pale basal band on hind tarsomere 2 and (3) pale bands crossing all four of the hind tarsal joints. It belongs to the *vanus* subgroup along with *A. ahomi* and *A. barbumbrosus*.
3. *A. vanus* There is considerable doubt whether it occurs in India. *A. vanus* of Wattal, Kalra & Rajagopal (1962) recorded in India is now generally regarded as a variety of *A. barbirostris* and not the same as *A. vanus* of Walker (Reid, 1968). Therefore it is not included for the present in the list of species occurring in India. *A. vanus* belongs to the same subgroup as *A. barbumbrosus* from which it can be distinguished by the largest phallosome leaflet not strongly curved and by pupal characters. Pupae have a trumpet differently shaped, without a secondary cleft or seams and hair No. 1 and 5 are not tufted.

Wattal *et al.* (1962) have reported that there are two distinct kinds of *A. barbirostris*, one with narrow scales and another with broad scales on the venter. Only those with broad scales are found in the western parts of the country, *viz.*, Punjab, Uttar Pradesh Terai, Maharashtra, and South India, except for one narrow scaled specimen from Yellapur (North Kanara). Both broad scaled and narrow scaled specimens occur in eastern India. In Andamans only narrow scaled specimens are found.

Distribution: India, Pakistan, Bangladesh, Nepal, Burma, Thailand, Malaysia, Laos, Kampuchea, Vietnam, N. Borneo, Kalimantan, Indonesia and as far as Moluccas and Timor, Sri Lanka and South China. The previous records in the Philippines are of other related species, such as *franciscoi, manalangi* or *vanus*. (Reid, 1962).

In India, it occurs in all zones. It has not been recorded from high elevations in the Himalayas, but has been found in the Darjeeling District of West Bengal. Nair, Jacob or Ramachandra Rao *et al.* did not record it in Kashmir or in high Himalayas. It is, however, found in the foothills (Issaris). In the Nilgiris it is recorded from Coonoor (1800 m) by Puri (1948). It becomes scarce in Gujarat and Rajasthan though specimens have been collected. It is found in the Andamans.

Prevalence

The species occurs throughout the year in the peninsular region when stagnant water is abundant particularly during and after the monsoon.

In Burma also it has a similar prevalence.

Adult Bionomics

Resting habits: *A. barbirostris* is generally regarded as a wild mosquito rarely

entering human habitations but specimens have been collected inside human and animal dwellings in small numbers in India. Christophers (1933) gives several records of indoor resting, from India, Thailand, Indonesia (Sulawesi) and Sri Lanka. Russell and Ramachandra Rao (1941) in a two years' study in Thanjavur District collected only 148 adults out of a total of 284,591 adults of all 12 species occurring in the area. In a magoon type of portable trap they collected only 33 females of the species as compared to 5,339 of *A. nigerrimus* collected during the same period. In the erstwhile Bombay State, 426 specimens were collected out of 595,579 specimens of all species found resting inside dwellings (Viswanathan 1950). In a two years' study of the distribution and seasonal prevalence of mosquitoes in Pune, Ramachandra Rao and Rajagopalan (1957) found only 3 females, one mdoors, one in outdoor shelter and one biting man outdoors, out of 2,948 anophelines collected.

Many more mosquitoes were collected in an open shed than in occupied closed huts in Assam (Yofe and Fox 1946). There are records of larger collections in houses and cattle sheds in India (Basu and Morjoribanks, quoted by Christophers, 1933), but in the general area of the latter's study Salsette Island near Bombay, the present author found only 12 specimens out of 4,000 of all species collected inside dwellings. Nair (1947) and Wharton (1950) have collected specimens occasionally in houses. In Burma, specimens have been collected resting in jungle during daytime.

Biting habits: *A. barbirostris* is generally regarded as a zoophilic species though it will bite man occasionally. In Malaysia, Reid (1961) reported that a calf was 17 times more attractive than two men. Adults have also been collected in Thailand biting man as well as cattle. According to Christophers (1933), it attacks and feeds readily on man in shade, in forest during daytime, in the Andamans. Reuben (1971) working in North Arcot District of Tamil Nadu found that the species was attracted to a bullock 38 times as frequently as 2 men. Russell and Jacob (1939) showed that none of the 18 wild females caught by them in the north of Madras had human blood. In the absence of cattle, *A. barbirostris* will readily bite man.

In Burma during the DDT spraying operations, in fixed catching stations, 96 per cent were collected biting cattle and only 4 per cent on human baits (Khin-Maung-Kyi 1971). On four occasions the females were found biting man in the jungle during daytime. In night collections, the species appeared early and continued biting upto 24.00 hours. Precipitin tests on stomach bloods have been very few in India, but in Malaysia Hodgkin found that out of 158 stomach bloods positive 107 (68 per cent) had human blood (Reid 1968), but such high rates appear unusual. There is no reliable information on other aspects of bionomics.

Larval Ecology

Breeding places: The full grown larvae is comparatively large with the size range 5 to 8 mm. It is greyish brown in colour and can be easily distinguished from the smaller sized greenish brown larvae of *A. nigerrimus*.

A. barbirostris larvae occur in many types of breeding places with stagnant or

flowing water but with vegetation or organic matter. Ponds, small ground pools, borrow pits, rice fields, slow running streams, shallow wells and even salt swamps are the known breeding places (Covell 1944). In Bengal Sen (1935, 1948) found that rice fields were not the preferred breeding places. In Thanjavur District, Russell and Ramachandra Rao (1940) found the species to prefer waste irrigation water in low lying places, including rice fields flooded freshly by irrigation water, but the larvae were found also in wells, tanks, growing rice fields, borrow pits, field channels and ditches. They collected the larvae in 97 breeding places out of 5,616 searches and only 539 larvae of the species occurred in a catch of 141,119 of all species. Russell and Ramanath Rao (1940) in the same area found that *A. barbirostris* occurred in rice fields only in the later stages of rice growth. They found that the species was associated with *A. nigerrimus* 41 per cent of times. In Hassan District of Karnataka, Brooke Worth (1953) recorded large numbers of *A. barbirostris* larvae but attributed it to the bias of sampling by collectors. Though he collected over 3,000 larvae, only 95 adults were found during a one year period. He actually collected 14,700 adults of all species. McArthur (1950a) observed in Borneo that *A. barbirostris* was found abundantly in rice fields when they were freshly flooded and gradually the number diminished as the plants grew. When irrigation was cut off at the end of the season and pools and puddles formed, the species re-appeared in numbers. The association of *A. barbirostris* with brackish waters has been recorded in the Andamans by Covell (1927).

Relation to Disease

Extensive series of dissections made in many parts of Southeast Asia have shown that while in India, Sri Lanka and Burma the species takes no part in malaria transmission, it is a vector of some significance in Sulawesi (Indonesia) where both gut and gland infections have been found (Horsfall 1972 and Wattal 1961). Covell (1944) has given records of several findings of infections in Southeast Asian countries, including a 10-13 per cent total infection rate in Sulawesi. Harinasuta *et al.* do not include it as a vector in Indonesia. Since the unravelling of the taxonomy of the *barbirostris* group by Reid (1962), some of the older records of plasmodial infections need to be reconsidered. Perhaps the records from western parts of Malaysia may be those of *A. campestris* and from Borneo those of *A. donaldi*. Much still remains to be clarified. According to Harrison and Scanlon (1975), because of its zoophily, exophagy and exophily, its potential for being a malaria vector in Thailand seems very low. Experimentally the species has a low susceptibility to *P. cynomolgi bastianelli* (Warren *et al.* 1963).

A. barbirostris is a recognised vector of human *filariasis* in Indonesia. In Kerala, India, Iyengar (1938) had shown that wild caught *barbirostris* were harbouring mature forms of *W. bancrofti*. Raghavan (1969) reviewing the vectors of human filariasis, had also recognised this as a vector. However, its role is a minor one compared to that of *Culex fatigans*. In Southeast Indonesia, in the Island of Flores, *A. barbirostris* has been found to harbour the Timor filaria larvae. Out of 129

females dissected, three larvae of first stage, five of second stage and 27 of third stage were found Apmasoudjono 1977. In Sulawesi it is considered to be a major vector of *P. malayi*, *A. barbirostris* has been recorded in that area to be most endophilic and anthropophilic.

A. barbirostris was reported infected with *P. malayi* in Thailand by Iyengar (1953). However, in view of the very recent clarification of the taxonomy of this group the exact status of *A. barbirostris* as a vector is not clear.

The potentiality of *A. barbirostris* as a vector of animal filariasis seems to be high and some of the earlier records presumed to be infections with human filaria parasites may in fact be due to animal parasites.

A. barbirostris has been found infected in nature with Japanese encephalitis virus in West Bengal (Chakravarty *et al.* 1975 and National Institute of Virology, Pune 1979). In view of the increasing recognition that anopheline mosquitoes may take part in transmission of virus diseases, these findings are of great significance.

Anopheles barbumbrosus Strickland and Chowdhury, 1927

Type locality: Nusa Kambangan, Indonesia.

Type: Not designated. Actually the name *barbumbrosus* was given by Strickland and Chowdhury to *A. barbirostris* var. *pallidus* of Swellengrebel (1919). The name *pallidus* Theobald (1901) for an *Anopheles* had already been occupied. One male from the type locality (Nusa Kambangan) is in the British Museum of Natural History, but according to Harrison and Scanlon (1975), due to lack of adequate characters to separate the adults of this species from those of *A. vanus* it is best to leave it as it is till the fauna of Indonesia is better known.

Taxonomy: The species is closely related to *A. barbirostris*.

Distinguishing characters: Adult can be distinguished from other forms by: abdominal sterna without white scales; apex of wing with lower pale fringe spot quite wide extending from vein 3 to vein 4.1. Larvae can be distinguished by outer clypeal hair with 12 to 36 thin attenuated branches usually slack (not stiff) and spread out (not broomlike).

Distribution: India, Nepal, Thailand, Malaysia, Sri Lanka, Indonesia (Sumatra), Kampuchea, and South Vietnam.

In India: Wattal *et al.* (1962) during their re-examination of the adult collections in the National Institute of Communicable Diseases discovered 75 adults of *A. barbumbrosus* previously labelled as *A. barbirostris* from the following places: Assam, West Bengal, Bihar, Madhya Pradesh, Maharashtra (Nagpur), Andhra Pradesh (Tirupati Hills), Karnataka (Belgaum and Bangalore) and Madras. Reid (1962) also recorded it from Nepal and Sri Lanka.

Ecology: This is mainly a forest dwelling species. Little information is available on pupae and larvae in India. Being a comparatively rare species, no information on adult bionomics is available in India, but in Thailand, Scanlon and Esah collected *A. barbumbrosus* females biting man at altitudes between 260 and 1370 metres. Harinasuta *et al.* (1971) also report having collected specimens on human

bait. No information on daytime resting habits is available. Most of the larval collections made in Thailand are from ground and rock pools and stream margins. A few collections have also been made from other habitats such as ponds, holes in the ground, puddles, swamp margins, shallow wells, springs, rice fields, animal footprints etc. Even tree-holes, water pots and water jugs have contained the larvae.

Relation to disease: No information on relation to any human or animal disease.

Anopheles roperi Reid, 1950

Type locality: Kuala Kubu, Selangor, Malaysia.

Type: British Museum of Natural History, London.

Taxonomy: Originally recorded as a variety of *A. umbrosus* (Roper, 1914) and later regarded by Colless (1948) as a species under the *umbrosus* group in Borneo.

Distinguishing characters: Adult: a large sized mosquito. It has usually an accessory pale fringe spot on vein 2.2; base of veins 1 and 5 with pale scales; a pale basal band on the hind tibia; centre of mesonotum not uniformly pale.

Larva: Not easily distinguishable from closely related species. The nearest relative in India is *A umbrosus* but in Malaysia and Thailand *A. letifer* and *A. whartoni.*

Distribution: India, Thailand, Malaysia, Sumatra and Kalimantan and Kampuchea. Harrison and Scanlon (1975) state that *A. roperi* is known in India from Assam only (Also refer to Stone and Delfinado, 1973 and Reid, 1968). Harrison and Scanlon felt that many *A. umbrosus* previously identified in Assam could actually have been *A. roperi.*

Bionomics/Ecology: Nothing is known about the ecology in India. In Thailand and Malaysia it is known rarely to be taken inside houses, but adults will bite in the forest throughout the 24 hours with a peak in late evenings. (Moorehouse and Wharton 1965). MacDonald *et al.* (1967), working in Sarawak collected females biting man near open rice fields in the evenings. The species is known to feed on monkeys. In comparative studies made between collections at ground level and in a tree canopy, less than 10 per cent of the adults were captured in the canopy. The species breeds in wooded valleys. The larval habitats are shaded pools, swamps and ditches and flooded pools.

Relation to disease: There is no information in India. Though gut and gland infections have been found in nature in Malaysia, Wharton *et al.* (1963) have considered them to be of animal origin probably of mouse-deer malaria. Several natural infections with *Plasmodium traguli* have been found with heavy oocyst and sporozoite rates. At one time it was epidemiologically considered that *roperi* was a vector of human malaria in Malaya, but it is not confirmed. Sporozoites injected into rhesus monkeys and man failed to produce infections (Wharton *et al.* 1963). Bennett *et al.* (1966) were able to demonstrate oocyst development of a Cambodian strain of *P. cynomolgi* in this species.

There is no evidence that the species has any role in transmission of human filariasis. However, the natural infection of filarial larvae found is that of *Setaria* species reported by Wharton *et al.* (1963). Experimentally, it has been shown that *W. bancrofti* could grow upto the mature stage in *A. roperi.*

Anopheles umbrosus Theobald, 1903

Type locality: Pekan, Pahang, Malaysia.

Type: British Museum of Natural History, London.

Taxonomy: Stone *et al.* (1959) and Knight and Stone (1977) say that this name is a homonym by a few weeks of a variety of *Anopheles funestus* var. *umbrosus* Theobald (= *funestus* Giles) but as the name *umbrosus* has been used extensively it is contemplated that primary action will be requested by the International Commission to preserve the name for the widely used *umbrosus* Theobald of the Oriental Region. Harrison and Scanlon (1975) say that they had examined specimens which confirm the occurrence of the species in Assam though Reid (1968) had suggested that the records of *umbrosus* from Assam may actually refer to *A. roperi*. As with the *hyrcanus* and *barbirostris* groups, there has been much confusion regarding the name *umbrosus* and it is difficult to be sure about the specific identity of the earlier records. It is only after Reid (1959) that some clarity has been brought about. It is desirable to re-examine the material from India.

Twelve members of this rather heterogeneous group have been recognised (Reid 1968). They are regarded as sibling clusters. They are all very similar and not easily distinguishable except by minute differences. The twelve species of the groups are:

baezai	*brevipalpis*
brevirostris	*collessi*
hunteri	*letifer*
roperi	*samarensis*
separatus	*similissimus*
umbrosus	*whartoni*

Of these only *umbrosus* and *roperi* are recorded from India. The members of this group extend in their distribution from India (Assam and the Andamans) to the Philippines. Malaysia and Indonesia appear to be the centre of their distribution because 11 of the 12 species occur there.

Distinguishing characters: It is a fairly large sized mosquito comparable to *A. nigerrimus* and *A. barbirostris* with a dark appearance. Adults with unbanded palpi; pro-pleural setae present; costa without scattered white scales; no fringe spot on posteror margin of wing; pale tarsal bands narrrow and proximal; 1-6 setae and no scales on mesepimeron. Larvae can be distinguished by absence of palmate hairs, except on abdominal segments IV & V on which they are partially developed and unpigmented. Larvae with a long tapering stigmal process.

Pupa is distinguished, with a distinct targus on the trumpet; denticles on caudal margin of abdominal terga and extra large seta No. 9 on segment 5.

Distribution: India, Thailand, Malaysia, Indonesia, Philippines, 'Indochina'. In India, it has been reported from "Assam" and from the Andaman Islands, Christophers, 1933); but it has not been found in subsequent surveys.

Strangely there do not seem to be records of *A. umbrosus* in Burma.

Adult Bionomics

It is a species generally present in heavy forested areas. According to Christophers (1933) it has been described as occurring in Malaysia in houses close to the forest and that it can feed on man both in nature and experimentally. Little information has been collected in India but extensive studies have been made in Malaysia. Adults are collected mainly outdoors.

The species has been found to be a daytime biting mosquito, active throughout the daylight period in the forest shade with a peak just before nightfall and a small peak soon after daylight. Moorehouse and Wharton (1965) in Malaysia found the following numbers biting human baits at different times.

6.00 to 9.00 hours	152	
10.00 to 13.00 hours	45	462
14.00 to 17.00 hours	177	
18.00 to 19.00 hours	88	
20.00 to 05.00 hours	83	83

While for the entire hours of darkness from 20.00 to 05.00 hours they collected only 83 females, they captured 462 for the period of daylight from 06.00 to 19.00 hours. *A. umbrosus* therefore is among the few anophelines collected during the same studies. *A. letifer* was predominantly nocturnal but *A. roperi* and *A. donaldi* largely diurnal.

Reid (1961, 1968) has made extensive observations. He found the adults to enter houses and in a comparative study of adults biting man outdoors and in huts provided with window traps, he captured only about 14 per cent in the huts. A calf was more attractive than man. The species is also known to bite monkeys more at ground level than higher up on platforms (Wharton *et al.* 1964). However, man appears to be much attractive than monkeys.

When comparative studies were made of females biting at floor level in forests, 292 were found biting man and 31 on monkeys. When a comparison of biting monkeys at ground level and a canopy was made only 8 (28 per cent) were found in the canopy.

No precipitin tests on stomach bloods in India seem to have been done, but in Southeast Asia, Wharton (1953) summarizing the data, recorded that 90 per cent out of 333 had human blood. Obviously, *A. umbrosus* will readily feed on man. It can even be called more an anthropophilic mosquito rather than a zoophilic one.

There are no studies on longevity under natural conditions nor of dispersal. One laboratory observation by Green (1935) (quoted by Horsfall 1972) indicated that the females may live upto 20-59 days (average 38 days) in cages.

Larval Ecology

In Assam, according to Christophers the species was found breeding in stagnant shallow water in the forest. Muirhead Thomson (1942b) reports having collected larvae from jungle-pools in the forests in Assam. The breeding places surprisingly

had a low degree of pollution though the pools contained many rotting leaves and grass. In Malaysia where the species has been studied in greater detail the larval habitats are shaded pools and marshes, the water of which is stained from peatysoil. Similar observations have been made in Thailand. In Indonesia larvae have been found in slow running streams in deep forest. The breeding in brackish water in the mangrove swamps has also been recorded but they may not refer to true *umbrosus*, but to other related species. From all accounts *A. umbrosus* is a shade loving jungle species.

Relation to Disease

In evaluating the role of *A. umbrosus* in malaria transmission one should recognise that all dissections prior to 1950 should be considered with some doubt because of the taxonomical confusion which existed at that time. *A. umbrosus* was recorded at one time as a major malaria vector in Malaysia and also in North Borneo where quite a large number of gut and gland positives had been found. (See Horsfall, 1972 for list). Wharton *et al.* (1963) have now suggested that many of the infections might not have been of human plasmodia. The size of the oocysts suggested that the infections were those of *Plasmodium traguli*, a parasite of the mouse-deer, *Tragulus javanicus*, and that *A. umbrosus* rarely, if even, transmitted human malaria (Moorehouse, 1965). However, as *A. umbrosus* readily feeds on man, its positive role in human malaria transmission should be kept in mind.

There is a record of dissection of one *A. umbrosus* specimen in Jharia Coal Mines area in Bihar by G.R. Rao (1941) but the identification seems to be in doubt. The dissections made in India had been negligible, totalling six. (Wattal, 1961).

A. umbrosus has been infected in the laboratory with *P.c. bastianelli* (Warren *et al.* 1963), but its role in nature has not been assessed.

A. umbrosus plays no part in human filaria transmission. Wharton *et al.* (1963) failed to infect it with *W. bancrofti*. It could be a vector of animal filaria, particularly *Setaria* spp. (Wharton *et al.* 1963).

Anopheles balabacensis Baisas, 1936

Type locality: Balabac, Balaboc Island, Philippines.

Type: Institute of Hygiene, University of Philippines, Manila.

Taxonomy: Till Colless (1950, 1956) made a detailed study of this complex, the species occurring in India was being identified as *A. leucosphyrus*. All earlier records by Christophers (1933) and others refer to either *A. balabacensis* or *A. elegans*. True *A. leucosphyrus* is not now recorded as occurring in India. Colless (1956) examined the entire complex in detail and came to the conclusion that there were eight forms with distinct morphological characters. They were:

A. hackeri	Borneo, Malaysia, Sumatra.
A. elegans	India, Sri Lanka.
A. pujutensis	Borneo, Malaysia, Sumatra.

A. balabacensis	Philippines, Borneo, Malaysia, Java, Thailand, Indochina, Burma, India and Bangladesh.
A. leucosphyrus	Borneo, Malaysia, Sumatra.
A. leucosphyrus riparis	Philippines.
A. l. macarthuri	Malaysia, Borneo.
A. cristatus	Philippines.

The name *A. leucosphyrus* does not occur in the *"Check list of Mosquitoes of the Oriental region"* by Stone and Delfinado (1973) but occurs in the World Catalogue by Knight and Stone (1977).

One more has since been identified, *viz. sulawesi.* Revised keys to the adults have been given by Reid (1968).

It is essential to note that both wings should be examined. Keys for the males and larvae are not given by Colless but Reid (1968) may be consulted.

A. balabacensis has two sub-species, *viz. A. b. baisasi* in the Philippines, and *A. b. introlatus* in Malaysia & Thailand.

Recently, a new species, *A. dirus* Peyton and Harrison. 1979, has been created for the forms of *A. balabacensis* found in Thailand.

Anopheles dirus in India.

Dr. H.R. Bhat, National Institute of Virology, Pune, informs the present author (Personal communication, January 1981) that he has re-examined the collections of the specimens of so-called *A. balabacensis* from Shimoga and North Kanara Districts, Karnataka State, and that they tally with the description of *A. dirus* Peyton and Harrison, 1979. Recently adults have been collected both biting man and resting in cattle sheds. This finding makes it necessary to re-examine collections from all parts of India to determine: (1) the distribution of *A. dirus* in India and (2) what is the exact status of *A. balabacensis* in this country.

Distinguishing characters: The adult *A. balabacensis* is comparatively a moderate sized mosquito. Because of the numerous dark and white spots on its wings and also the speckling on the legs and the white band in the tibio-tarsal joint, the adults has bright, highly spotted appearance. The specific characters distinguishing it from *A. elegans* are: presector dark spot on vein 1 with one or more pale interruptions; middle and/or preapical dark marks usually with two or more pale interruptions, at least on one wing; apical band of hind tibia without a ventral dark stripe. It is also distinguished from *A. elegans* by the proboscis being distinctly longer than hind femur and often longer than the palp. (See Kalra and Wattal 1962).

Distribution: India, Nepal, Bangladesh, Burma, Thailand, Indonesia (Java), Malaysia, Kampuchea, North Borneo, South China, Taiwan, Philippines. (Sri Lanka?)

In India: in the eastern and western zones. Assam and neighbouring areas are the main areas of prevalence. Most probably all the records in the eastern parts refer to *A. balabacensis.* Forms in South India could be both *A. balabacensis* and *A. elegans.* Occurs in the Andamans but not in Lakshadweep. The form in Sri Lanka is *A. elegans.*

Kalra and Wattal (1962) re-examined the adults of this group in the collections at the N.I.C.D., Delhi, and found that out of 92 adults from India, 64 were *A. balabacensis* and 28 *A. elegans*. The former species occurred in many parts of India including the Andamans, Assam, Kerala, Tamil Nadu, Karnataka, Maharashtra, Punjab, Tripura and West Bengal, but the latter species only in Kerala, Karnataka and southern tip of the coast of Maharashtra. The sole specimen of *A. leucosphyrus* in the collections from Sri Lanka was found to be *A. balabacensis*. The species has been subsequently found in several localities in Karnataka. Recently the present author examined a small collection at the National Institute of Virology, Pune and found that all specimens preserved from North Kanara and Shimoga Districts were *A. balabacensis* and none was *A. elegans*. More intense collections in those areas are required. Nair (1973) recorded "*A. leucosphyrus*" from Kashmir during 1963. If confirmed this would be the western-most record. A single specimen has also been recorded from Kasauli (Himachal Pradesh) by Kalra and Wattal (1963). However, the species has not been found subsequently in any other surveys in the area.

Prevalence

It is a species prevalent in wet forested areas, at altitudes ranging almost from at sea level (Savantwadi) to the hills upto 1,000 metres or more. Its season of greatest prevalence is during the rainy season. How it persists in the dry season is not known. Sometimes it may be the most abundant species in parts of the eastern zone.

Adult Bionomics

Resting places: *A. balabacensis* is generally regarded as a wild species infrequently present inside houses and cattle sheds during daytime. There are early records mentioned by Christophers (1933) of a few specimens collected inside houses. In Burma it was mainly an outdoor rester and a few specimens were collected in day time inside houses in the bed-nets occupied by people. However all but one of the 129 mosquitoes collected inside houses at night had fresh blood, indicating that members of this species left soon after feeding for outdoor shelters (Macan 1948). In Burmese jungles it is extremely difficult to find adults in human habitations during daytime. Adults can be collected at night only on human bait collections (Khin-Maung-Kyi, 1971).

In Tirap District of Arunachal Pradesh, which borders Burma, detailed studies were made by Sen *et al.* (1973) on the bionomics of this species in 1969. This was an area where continuous malaria transmission was going on in spite of DDT spraying. Actually there was a increase in the number of malaria cases detected from 1964 to 1969 (from 784 to 4,114 cases). In human biting collections in which 14 species were collected, *A. balabacensis* constituted 1,828 adults (approximately 60 per cent) of the total of 3,047. Indoor biting collections showed very low densities. Inside dwellings all anophelines species together amounted to only 3.43 per man-

hour and the density of *A. balabacensis* was only 0.18 per man-hour. Outdoor shelters were searched for 216 hours, but only one each of *A. balabacensis* and *A. maculatus* was found. Obviously, the searches were inadequate.

McArthur, in his studies of the mosquitoes of Borneo, found that specimens could be found inside houses only on particular nights. Outdoor resting adults on the banks of streams have been reported.

Therefore, *A. balabacensis* is largely an out-door resting species.

Biting habits: Sen *et al.* (*loc. cit.*) found the species from March to October with a peak in September when *A. balabacensis* constituted over 60 per cent of all species collected. Its average man-biting rate was as high as 11 per day. Actually in one area, Namchik, the man-biting rate ranged from 21.6 to 35.2 per day in collections made in September, i.e., as many as 35.2 *A. balabacensis* were found biting one man on an average, a rate of biting rarely exceeded by any other Indian anopheline. Most of these night collections were made indoors in the usual types of dwellings, made of bamboo and mud as well as in dormitories constructed out of split bamboos with galvanized iron roof, indicating a considerable degree of endophagy. The other species found biting man were *vagus*—324, *kochi*—234, *philippinensis*—161, *maculatus*—146, *barbirostris*—138, *aconitus*—99, *annularis*— 28, *nigerrimus*—43 and others such as *jeyporiensis, pallidus, subpictus* and *varuna* in very small numbers.

The Burnihat area in Assam (Meghalaya) was studied by workers of N.I.C.D. (N.I.C.D. Annual Report 1970) in 1970. Out of 7,511 females of 18 species they found only 69 *A. balabacensis, A. philippinensis* constituted 1,864 specimens being next only to *A. vagus* (1,995). A very interesting finding was that no specimens of *balabacensis* and only 13 *philippinensis* were collected by hand catches during day time. On the other hand, 30 *A. balabacensis* were collected in the traps and 34 in biting captures, that too mostly outdoors. The time of biting at night was as follows:

20.00—22.00 hours . .	5
22.00—24.00 hours . .	12
24.00—02.00 hours . .	10
02.00—04.00 hours . .	7

confirming that though *A. balabacensis* does feed throughout the night, it was predominantly a midnight biter.

A point of great interest was that no adult of *A. minimus* was collected either in houses or traps or in biting collections. Obviously the species had disappeared over a large part of its domain. (See also under *philippinensis*). Rajagopal (1979) has also reported another one night study of anophelines biting at night on two men during which 104 anophelines of 6 species were collected at a locality in Mikir Hills, Assam, in a thatched field hut used for watching the crops at night. They were *A. balabacensis* 86, *A. aconitus* and *A. maculatus* 6 each, *A. philippinensis* 3, *A. splendidus* 2 and *A. kochi* 1. The times of collections and numbers collected of *A. balabacensis* were: before 22.00 hours—6; between 22.00 and 02.00 hours—65, and 02.00—02.30 hours—15.

In Thailand, *A. balabacensis* has been found to be highly anthropophilic and endophagic (Scanlon and Sandhinand 1965). The species was also found to feed on monkeys. The females rested in the forest vegetation during the day and congregated in the vicinity of human dwellings after dark just before entering houses. Eyles *et al.* (1964) working in a neighbouring Khemer region of Kampuchea, also noted the outdoor resting habits which minimised the possibility of controlling or reducing malaria where this species was a vector.

In Thailand, Ismail *et al.* (1974) have added considerably to the knowledge on *A. balabacensis*. Working in a rural area in Pitsmuloke Province of North Thailand they made many interesting observations. In *A. balabacensis*, in the earlier part of the study in July 1970, the indoor biting was about 4 times more than outdoor biting (44.4 and 11.82 per man per night respectively). In September the difference was only about 1.5 times and in October the proportion was nearly equal and in November there was indeed a reversal, the biting density outdoors exceeding that of indoors by more than three times. This seasonal difference in endophagy and exophagy was attributed to the influence by rainfall and the site of indoor collections.

In Thailand, *A. balabacensis* was comparatively a late night biter during the maximum breeding season, with a tendency to earlier biting towards the end of the season. The pattern of biting cycles was very similar whether it was indoors or outdoors. The peak activity of *A. balabacensis* in Thailand was at about 23.30 hours with numbers gradually increasing till then. Subsequently biting activity decreased. In Burma, the period of biting varied from place to place; but the main biting time is during the second and third quarters of the night, 21.00 to 03.00 hours (Khin-Maung-Kyi, 1971). Other studies on night biting showed that the peak biting was between 24.00 and 01.00 hours (Macan, 1948). All these observations indicate that *A. balabacensis* bites mainly in the middle part of night.

Based on over 6,500 dissections of female *A. balabacensis* between July and November, 3,300 (31%) were found parous, giving a probable daily survival rate between 0.835 and 0.865 in different years. There were no differences in parous rates between outdoor and indoor resters. Ismail *et al.* came to the conclusion after use of experimental huts, verandah trap huts, portable trap huts, etc. that *A. balabacensis* can enter compact structures, in search of blood meals; unlike *A. minimus;* but above 95 per cent of *A. balabacensis* leave the huts after taking the blood meal.

In dealing with the early literature on bionomics of the species one gets considerable doubt about the findings because it was only in 1956 that the taxonomic status of the species became clear. The earlier records may refer to any one of the other species of the group now recognised. However, in relation to the Indian forms found in Assam and Bengal, there is no doubt that the species dealt with by the authors was *A. balabacensis.*

Host preferences: Ramsay *et al.* (1936) found an anthropophilic index of 75 per cent. Comparisons of biting catches on man and cattle, both indoors and outdoors, in Southeast Asia showed that 42 per cent bite indoors and 84 per cent of all females collected were biting man. One calf attracted 12 while two men attracted 64 females. (Reid 1985, Table 14). Results of precipitin tests (WHO in Sabah, Borneo)

showed 94 per cent with human blood. Therefore, there is no doubt that the species is preferentially a man-biter.

Laboratory colonization: A laboratory colony of *A. balabacensis* was successfully raised by Eash and Scanlon (1966) in Thailand and it was reported to have had more than 39 generations without special techniques in cages of 60 cms x 60 cms x 60 cms. However, there appears to be some strain differences in the amenability to colonization.

Flight habits: Very little reliable information exists on the flight and dispersal patterns. Most workers regard 0.8 kms as the normal flight range and dispersal to be about 1.6 kms.

Larval Ecology

A. balabacensis is a pool breeder in the jungles. Christophers has given references to its breeding in pools by the side of rocky streams, in disused wells, in rain-water in borrowpits and in nullahs in densely shaded foliage. Krishnamurthy (1961) in his review expanded the observations. In the Digboi area of Assam, Clark and Choudhury (1941) have found this species breeding in small collections of stagnant water in the open jungle. They were small pools, in marshy areas, such as those made by the footprints of elephants. In no case were larvae found in completely unshaded areas or in running water; neither were they found in partially shaded or open tanks unlike other species commonly breeding in such places like *A. vagus, A. philippinensis* and *A. kochi.* All breeding places had rotting vegetation and often turbid water, sometimes fouled by buffaloes.

In Thailand, Ismail *et al.* (1974a) found three types of breeding places of *A. balabacensis*:

(a) Shallow seepage pools at the sides of seasonal water courses, partially shaded and with rather clear water and decaying foliage at the bottom. Such streams were located inside the forest and also near the village houses.

(b) Similar shallow pools as a part, but at the sides, of permanent streams. The location of these pools to change during the rainy season according to the water level in the stream.

(c) Cement basins located in the verandah trap huts, for protection against ants, containing clear water, but with organic matter at the bottom and partially shaded.

While the first two of the above types are in agreement with the experience in India, the third type, viz. breeding in ant-guards inside houses is unusual and merits further attention. In a recent study in southeast Thailand, Wilkinson *et al.* (1978) found that *A. balabacensis* could only be found in the forests. While some malaria transmission indoors probably occurred in the villages during the rainy season, the risk of acquiring malaria was highest in the forest. Oviposition occurred throughout the year in wet areas but larvae could be collected only near villages.

In Burma, according to Khin-Maung-Kyi (1971), the species is capable of breeding in a great variety of stagnant waters in the jungle but never exposed to sunlight.

All observations therefore indicate that *A. balabacensis* is a forest species breeding in shaded stagnant ground pools.

Eggs: An interesting observation made in Thailand by Wilkinson *et al.* (1978) was that eggs of *A. balabacensis* "when deposited on most soil at margins of ground pools remained viable long enough to survive intervals between rains in the monsoon season but there was no evidence that they could survive the dry season." Iyengar (1938) described eggs of *A. balabacensis* (then known as *A. leucosphyrus*) for the first time collected from Darjeeling District, West Bengal and gave the following measurements and descriptions:

They closely resemble the eggs of *A. tessellatus* but with gerater breadth of the float and by the upper surface of the egg being more concave.

Measurements of *A. balabacensis* eggs.

	Max.	Min.	Ave.
Length	0.56 mm	0.51 mm	0.54 mm
Breadth	0.18 mm	0.15 mm	0.16 mm
Number of float ridges	20	17	18

Relation to Disease

A. balabacensis is perhaps one of the most efficient vectors of human malaria. It is very strange that the role of this species as a malaria vector had not been recognized till very recently. In fact, Christophers (1933) stated "It is not thought to play any part in malaria transmission in India owing to it's rarity except as a jungle species". There is no doubt that, in the presence of *A. minimus*, which was regarded as the primary vector in most places in north eastern India, the role of *A. balabacensis* had been overlooked. It was only the persistence of malaria in the eastern hill regions after a good control of *A. minimus* that led to the recognition of its important role. Today perhaps it is the most important vector in the hilly regions of Assam and neighbouring hill states.

Clark and Choudhury (1941) noted its importance in the Digboi area of Assam and found an infection rate between 3.1 and 4.9 per cent during 1938 and 1940. However, they still regarded *A. minimus* as the most important vector, with infection rates of 3 to 3.2 per cent. Clark and Choudhury were the first to report infections in *A. balabacensis* but they quote that one Crawford in 1938 had noted natural infections in the species. Out of 859 specimens which Clark and Choudhury dissected eight gut and 19 gland infections were found. The species might have played along with *A. minimus* a major role in the high incidence of malaria among British, American, Indian and Japanese troops in the Burma front in the Second World War.

Sen *et al.* (1973) found in Tirap area of Arunachal Pradesh in the month of September three gland infections among 1,811. *A. balabacensis* dissected and none of other 10 species of anophelines dissected (total 3326) showed any positives. Incidentally they found no specimens of *A. minimus* in that area.

In Burnihat (Assam/Meghalaya) Rajagopal (1976), during his studies in 1966 found *A. balabacensis* to be rather rare in biting collections on man or cattle, only one individual having been collected. It did not seem to have any role in malaria transmission though it had been suggested to be a vector earlier. In that area it was *A. philippinensis* which was the vector. The very low prevalence of *A. balabacensis* in that area needs further investigation.

In other countries of Southeast Asia the species has been long known as a malaria vector of importance. But, many of the dissections made might have been of other members of the *A. leucosphyrus* group which became separately recognised only after Colless's work in 1956. Many dissections have been made in Borneo by McArthur and others, in Sumatra and Java by the Swellengrebels, Doorenbos and others and smaller numbers have been dissected in 'Indochina' and Burma.

Covell (1944) has summarized the information on "*A. leucosphyrus*" as follows:

> "Roper (1914) suspected *leucosphyrus* to be a malaria carrier in Borneo on epidemiological grounds. Bais (1919) dissected 235 specimens of which four had gut infections (1.7 per cent) and considered to be an important vector in the Dutch East Indies. In more recent years it has been found infected in several localities in Sumatra. Doorenbos (1931) reported 504 dissections in that island with an infectivity rate of one per cent. Stoker (1934) recorded seven infections out of 110 specimens caught in houses at Sarang-Tioeng, an island of the west of Borneo, and Galvao (1934) found one out of 13 infected in a locality in East Borneo".

Many of these records undoubtedly refer to *A. balabacensis* which has the widest distribution among all members of this group. McArthur's dissections in Borneo have totalled over 10,000, with 128 gland infections and 95 gut infections. In Sarawak 30 gland infections have been found out of 7,568 dissected (Zuluetta and Lachance, 1956).

Khin-Maung-Kyi (1971), summarizing the work in Burma, has shown that Lal Maung Thein (1958) dissected 2,061 specimens in Cochin State of which 58 showed sporozoites. He also refers to his own dissections at the Madaya Town in Mandalay District where, out of 204 specimens dissected during 1959, there was a sporozoite rate of one per cent. Khin-Maung-Kyi concludes that *A. balabacensis*, is a major vector during the monsoon months wherever it occurs in thickly forested areas of Burma.

Among other recent dissections record are those of Scanlon and Sandhinand (1965) in Thailand and Eyles *et al.* (1963) in Kampuchea. In Thailand, Ismail *et al.* (1974) dissected 3,573 specimens in northern Thailand and found 16 sporozoite positives or 0.5 per cent as against 0.13 per cent in *A. minimus* (dissected 23,252, sporozoite positives-3). It can be noted that the infection rates are not as high as those recorded for *A. fluviatilis* but *A. balabacensis* should be regarded as a vector of significance wherever it occurs.

A. balabacensis is known as a vector of monkey malaria and Reid (1968) cautions that the natural infections found with this species should be interpreted care-

fully because some of them may belong to monkey plasmodia. In laboratory experiments on the transmission of monkey malaria parasite *P. knowlesi*, Collins *et al.* (1971) found *A. balabacensis* to be a better vector for transmitting the parasite than *A. atroparvus* and *A. quadrimaculatus.*

Control

A. balabacensis is extremely difficult to control by anti-larval measures because of its breeding habits. Clearing of trees and vegetation, however, seems to prevent breeding (McArthur, 1954), because of direct sunlight reducing breeding by 90 per cent. However, the effect seems to be an indirect one is caused by change in the ecological conditions, probably changing the fauna and flora, because reduction in breeding is gradual. Originally it was a seepage breeder in the jungles but as man entered the environment, increased breeding occurred as was found during the military operations during the Second World War.

The species is also difficult to control in the adult stage because of the marked habits of exophily. Malaria has persisted in its areas of prevalence in the eastern hilly regions of India in spite of vigorous DDT spraying activities under the NMEP. There is no evidence that it has become resistant to DDT.

However, Ismail *et al.* (1974b) in Thailand studied the effect of DDT spraying with dosage of 2gm/m^2 and found that malaria transmission, though not interrupted was well controlled. Significant decrease in the population densities of both *A. balabacensis* and *A. minimus* occurred; but with *A. minimus* after a marked fall in one season the density increased in the second. Contact with man was much lower both indoors and outdoors. Studies on biting cycles showed that biting occurred earlier than before. The excitorepellant effect of DDT seemed to stimulate a high number of *A. balabacensis* to leave without taking a blood meal. In the postspraying observations, *A. balabacensis* showed a total decrease estimated at 37.5 times in indoor population and 17 times in outdoor populations. Calculations on the basis of the reproduction rate as proposed by Macdonald (1957), indicated that with *A. balabacensis* each primary case of malaria led to new infections as follows:

	Pre-spraying 1971	Post-spraying 1972	1973
Indoor population	318	32	9
Outdoor population	305	89	20

These data suggest that *A. balabacensis* is affected by DDT spraying directly or indirectly. No such observations have been made in India.

The part which *A. balabacensis* plays in non-human malaria or in human and animal filariasis is not known. However, *A. balabacensis* has been infected in the laboratory with *Plasmodium knowlesi* (Collins, *et al.* 1971).

Addendum

A few articles of importance have recently appeared which have relevance to the identification and distribution of *A. balabacensis.*

1. Peyton, E.L. and Harrison B.A. (1979), (*Mosquito Systematics* 11, 40-52) have described a new species, *A.* (*Cellia*) *dirus*, among the leucosphyrus group in Thailand. According to them, a detailed examination of the adult,larva and pupa warrants recognition of the Thailand form, which was hitherto being described as *A. balabacensis balabacensis*, as *A. dirus*. Probably in all the major studies the form till now referred to as *A. balabacensis* in Thailand was *A. dirus*. *A. dirus* is probably a major malaria vector in that country. This study has again thrown the taxonomy and disease relationships of the members of this group in other parts of Southeast Asia into doubt, requiring re-examination of the material from all parts of the region.

While the occurrence of *A. dirus* in Thailand is confirmed, the authors say that pending further study the species "probably represents most records of *'balabacensis'* in mainland Southeast Asia north of latitude 8°."

2. Baimai, V. Harrison, B.A., and Nakavactara, V. (1980), (*Proceedings of Entomol. Soc., Wash.*, 82, 319-328) have studied the salivary gland chromosomes of *A. dirus*. They state that *A. dirus* is not related either to *A. farauti* or *A. tessellatus,* two other members of Neomyzomyia series. This confirms the identity of these species.

3. *A. balabacensis* s.l. (*A. dirus*). In recent studies in Sylhet District of Bangaladesh (Rosenberg and Maheswary, 1982 and Rosenberg, 1982), much new information on the bionomics and role as a vector of *A. dirus* (*A. balabacensis* s.l.) has been obtained. A sporozoite rate of 3.65% was found among *A. dirus* found biting man. Nearly 85% of all man-biting anopheline species collected belonged to this species. The only other species found infected was *A. annularis* (one sporozoite infection). Study of parity conditions indicated that about 31.3% of *A. dirus* had lived long enough to become infective. Before the use of DDT the species was found biting both indoors and outdoors, but after the use of DDT the biting took place more frequently outdoors. Feeding pattern was influenced by the phases of the moon; the peak outdoor feeding was "sharpest and earliest" at the first quarter and was later as the moon rose later. The effective flight range was about 1.5 km. During the dry season breeding was found in a perennial stream about 1.5 km. from the place of adult collections and none of the other four types of breeding places available, viz. artificial containers, natural containers, wells and seepage and 'Stock tanks' was breeding the species. In the rainy season (April to October), all the above breeding places were present and in addition three other types of breeding places were available and only two of them viz. "Turbulance pits" in canals and puddles in paths supported nearly all breeding of the species which took place. Rain water pools were rarely positive as they became dry very

soon unless replenished by rain at very frequent intervals. A very interesting observation made was that larvae could leave pools and crawl overland for about 53 cm to another pool.

Anopheles elegans James, 1903

Type locality: Karwar, Karnataka State.

Type: British Museum of Natural History, London.

Taxonomy: Long regarded as a synonym of *A. leucosphyrus,* it has been given the status of a full species by Colles (1956).

Distinguishing characters: Adults of *A. elegans* can be distinguished from *A. balabacensis* by; proboscis longer than fore femur; presector dark spot on vein 1 without any pale interruptions; the presence of a dark longitudinal stripe ventrally on the apical white band on hind tibia. It would be desirable to see wings and legs on both sides carefully and check with the keys given by Colless (1956) and Kalra and Wattal (1962).

Distribution: India, Sri Lanka.

In India: It occurs along the western ghats of the southern states of Tamil Nadu (Nilgiris and Anaimalais), Kerala and Karnataka (North Kanara, Coorg and Hassan Districts). The present author has seen a specimen collected in Chittoor District, A.P. in a hilly area by Mr. S. Ramanujam when working in the Virus Research Centre Field Station at Vellore, 12 miles away. In several places in Western Ghats both *A. balabacensis* and *A. elegans* are recorded (Kalra and Wattal 1962). In collections made in Shimoga District (Bhat's Collections) and old specimens collected in North Kanara District, the present author did not find any *A. elegans.* All specimens were of *A. balabacensis. A. elegans* is presumably scarcer of the two. Does not occur in the Andamans or Lakshadweep.

Adult and Larval Ecology

Being a comparatively rare species its breeding habits are not well known but they have been collected in southern India in tree holes. It has also been found in rock pools as well as in ground pools in habitats similar to those of *A. balabacensis.* The species occurs in the deep jungle. In Nilgiris, southern India, Choudhury *et al.*, (1963) were not able to collect any *A. elegans* adults in houses but only from outdoor resting places such as dark damp shady places in arecanut gardens and shrubs near the base of arecanut trees. Sufficient number of males were also collected in similar situations indicating that the species was breeding in the stagnant waters around the bases of the arecanut trees. In the wooded areas of Hassan District Brooke Worth (1953). recorded one adult and 16 larvae of *A. elegans.*

Relation to Disease

There is no evidence of *A. elegans* being involved in transmission of human malaria. Choudhury *et al.*, (1963) found in the foothills of the eastern side of

Nilgiris that out of 84 *A. elegans* females dissected 19 showed sporozoites in the glands. The sporozoites were slender and their length varied from 8 to 14 microns. Oocysts were found in eight other females. Uninfected *Macaca radiata* monkeys were inoculated with sporozoites from four of these infected mosquitoes. All monkeys developed mixed infections of *P. cynomolgi* and *P. inui* after prepatent periods from 8 to 13 days. This study definitely established *A. elegans* as a vector of monkey malaria. Further studies of feeding of *A. elegans* females on infected monkeys showed that mosquitoes fed on them became infected. *A. stephensi, A. fluviatilis* and *A. tessellatus* also become infected and develop sporozoites when fed on infected monkeys.

Nelson (1971) showed that in Sri Lanka sporozoites from naturally collected *A. elegans* when inoculated into a rhesus monkey, resulted in infection with *Plasmodium shortti.*

Anopheles kochi Grassi, 1899

Type locality: Padang (Sumatra), Indonesia.

Type: Zoologisches Museum der Humboldt Universitat, Berlin.

Taxonomy: There are three synonyms: *ocellatus* Theobald, 1901 Perak, Malaysia; *flava*: Ludlow, 1908 Quezon, Luzon, Philippines; and *halli* James, 1901 Sylhet (now in Bangladesh).

Distinguishing characters: The adult is medium sized. Presence of conspicuous abdominal hair tufts on the ventral side of segments II-VII, visible to the naked eye; female palpi with four pale bands; vein 6 with not more than three dark spots. In general colouration similar to *A. subpictus* or *A. vagus.* The species cannot be confused with any other because of the abdominal tufts.

Distribution: Extensively prevalent in the Oriental region commencing from India, Nepal, Bangladesh, Assam and neighbouring hill states through Burma, Thailand, Indonesia, South China, the Philippines, Malaysia, N. Borneo, Kalimantan, Sulawesi and Moluccas. Not present in Sri Lanka, Taiwan or Hong Kong. There is a report of the presence of *A. kochi* in Nepal (Shreshta, 1966). This perhaps represents the western most point of its range of distribution.

In India, it is present in Sikkim, West Bengal and Assam and neighbouring hill states.

In their surveys of the Himalayan region, Ramachandra Rao *et al.,* 1973 did not find any specimens in the foot hills of the Himalayas extending from Kashmir to Sikkim.

Prevalence

In India it is not an uncommon species in the jungle areas of Assam and neighbouring states. It is more abundant in Thailand and Malaysia. Sometimes it is the most dominant species in a locality as in the sub-Himalayan regions of Sikkim and West Begal (Varma and Mahadevan, 1970).

Adult Behaviour

Resting habits: The adults rest mainly outdoors and only occasionally they are taken in houses. It is found more at night times in houses which it enters for feeding. The nocturnal man-hour density of the species found resting in houses was 0.44 per man hour in the Tirap region (Sen *et al.*, 1973). Though they collected no outdoor resting specimens, 234 out of 3047 anophelines collected at night biting man were *A. kochi*. Though Christophers (1933) had stated that it is a fairly common species inside dwellings and is also found in the jungles, the numbers collected inside dwellings now-a-days are negligible.

In Malaysia, the species has been found resting in jungles or on short vegetation in the vicinity of labour quarters (Wharton, 1950). In Burma, too, in recent years, no specimens have been collected in houses and cattle sheds, etc., but only outdoors. Khin Maung-Kyi (1971) feels that it is probably due to the effect of DDT operations. On the whole, *A. kochi* is an outdoor rester, but comes into houses at night for feeding.

Biting habits and host preferences: The females bite man readily as shown by Sen *et al.* (*loc. cit*). Comparative figures for biting on cattle and on man are scarce in India. In Burnihat area of Assam/Meghalaya, Rajagopal (1976) found five females biting man outdoors in 12 man-nights and only one biting man inside houses in 8 man-nights, while he collected 82 females biting cattle in 46 man-hours. In Malaysia it feeds chiefly on cattle to which it is attracted more than to man (Reid, 1961). It is also attracted to monkeys (Reid and Weitz, 1961).

Reid (1968) made a summary of all biting catches in Southeast Asia (in his Table No. 14) and only one per cent was found biting man when simultaneous opportunity existed to bite a calf. In a total of 31 nights, 1,228 females were caught on a calf and only 13 on two men. When comparative studies were made, 41 were caught on a monkey at ground level and only 10 on a man while 7 were collected on a monkey on a tree canopy.

Precipitin tests in Malaysia and other countries have been compared and the percentages which had human blood were:

Assam:	1 per cent (Ramsay and others, 1936)
Indochina:	6 per cent (Toumonoff, 1936)
Indonesia:	4 per cent (Walch, 1932)
Malaysia:	16 per cent (Hodgkin, 1932, 1935). (Tabulated from Reid, 1968)

In specimens collected outdoors by different workers, 102 out of 110 stomach bloods examined had cattle blood, 1 goat blood, 2 pig blood and even 1 bird blood, but not a single one with human blood was found. Four had blood of unidentified animals. On taking all factors into consideration, *A. kochi* may be considered among the least attracted to man. In Burma, the species has been found feeding in recent years on man only twice. Therefore, Khin-Maung-Kyi regards it as a jungle species and a cattle feeder.

Biting time: In Burma, the species is regarded as an early biter before midnight, but preferably in the first quarter. Using cattle baits, nine have been collected in the first quarter, two in the second quarter and none in the third and fourth quarters.

Flight and dispersal: Being a non-vector of human malaria, it has not received much attention in this regard in India. In other countries such as Indonesia flights upto 1000 metres from a release point have been recorded. (Lallemont quoted by Horsfall, 1972).

Larval Ecology

A. kochi breeds in shallow muddy collections of water. Ground pools with or without grass, hoof-marks, fallow rice fields, etc. are its breeding places. It generally occurs in the open but rarely in shade. Sometimes it has been found in drains in the jungles. It may even sometimes occur in artificial containers though uncommonly. In general its breeding habits appear to be similar to those of *A. subpictus* but occurring more in the hills and foot hills.

Relation to Disease

Though infected specimens have been found several times in nature including in Assam, the species is not regarded as an important vector of human malaria. Infected specimens have been found in India, Sumatra and Java. In India Wattal, (1961) has recorded 7 series of dessections and positives were found by Strickland, (1921) in Cachar District, (one infected specimen both gut and gland). Ramsay (1930) in the same area detected two infected specimens, (both gut and gland in 2,094 dissections) and Manson and Ramsay (1933) found one infected specimen (gut only) in Jorhat.

Anopheles tessellatus Theobald, 1901

Type locality: Taiping, Perak, Malaysia.

Type: British Museum

Taxonomy: Several synonyms but none occurs in India. There are two subspecies, *orientalis* Swell.& Swell. deGraff, 1919 and *kalavar* Stoker and Waktoedi, 1949. Neither occur in India. This species was often called *A. punctulatus* and for a time also referred to by Indian authors as *A. thorntonii*. All collections after 1933 are recorded as *A. tessellatus*. True *A. punctulatus* Donitz occurs in the West Irian and neighbourhood.

Distribution: Very extensively occurs in the Oriental region. India, Nepal, Bangladesh, Sri Lanka, Maldives, Burma, Thailand, 'Indo-china', Malaysia and Borneo, South China, including Hong Kong, Taiwan, the Philippines, Indonesia, as far east as Sulawesi and Moluccas. Records from West Irian (New Guinea) may be due to misidentification.

In India, it is widespread but becomes scarce in north western parts. The species has been found in Gujarat.It is present in the Andamans as well as in Lakshadweep.

Distinguishing characters: A medium sized mosquito, rather pretty to look at because of the speckling of legs and palpi, three broad white bands covering the apical half of the palpi and a narrow basal white band on the basal half of the palp and a highly spotted wing and speckled legs.

Adult: The palp of the female has three broad and a short pale band. The apical half of the proboscis is also pale. Legs speckled. Hind tarsi with narrow pale bands and wings with fringe spots against all veins.

Larvae: Outer clypeal short, stout and wide apart; inner clypeal appears frayed with 2 to 5 short branches.

From the close relatives of the group *Neomyzomyia* the adult can be distinguished by the absence of certain characters. *A. kochi* has a number of conspicuous tufts on the ventral side of the abdomen; *A. balabacensis* and *A. elegans* have a wide white band on the tibio-tarsal joint.

Prevalence

There are few studies on the seasonal prevalence of *A. tessellatus*, as it is comparatively an uncommon species though widespread in India. Though in small numbers, Russell and Ramachandra Rao (1941a) found it mostly in the cooler months from October to February. In the Malnad area of Old Mysore State, Brooke Worth (1953) found that the peak abundance was during southwest monsoon. The species was more prevalent in the low rainfall area than in intermediate and high rainfall areas. However, the species exhibited a relatively low tolerance to heat and dryness. In Nilgiris the species was prevailing in the East Nilgiris and absent in West Nilgiris (Russell and Jacob).

In Lakshadweep Islands also *A. tessellatus* and *A. subpictus* are known to occur. In 1974 Roy *et al.* found *A. tessellatus, A. subpictus* and *A. varuna*. Again in 1978 Roy *et al.* (1978) have reported that apart from *A. tessellatus* they also found *A. varuna* in Chetlet Island.

Adult Bionomics

Resting habits: Adults have been collected in houses, mixed dwellings and cattle sheds, in equal numbers in Pattukottai. In a period of three years Russell and Ramachandra Rao (1941a) found 158 adults inside dwellings out of over 200,000 of all species collected. Therefore, it was of some interest that they collected 61 adults (male 23, females 38) resting on the walls of wells, all in one month (December 1939). 25 wells were examined and 17 yielded *A. tessellatus* adults. Only 33 adults of other anopheline species were found. There was no breeding of *A. tessellatus* in any of the wells nor had the larvae of this species been found in wells in a three years study (Ramachandra Rao and Russell, 1940). Obviously, wells provided some combination of factors which made them specially attractive to *A. tessellatus* adults.

In Maldive Islands adults have been collected within dwellings (Iyengar *et al.*

1953) abundantly. There the species was resting particularly on the lower part of the walls of houses. The only other species found in the islands was *A. subpictus*. It has been reported that *A. tessellatus* had disappeared in Maldives after DDT spraying.

Biting habits: According to Reid (1968) adults bite cattle in preference to man. In Malaysia in 1961 he found that 20 times more *A. tessellatus* were attracted to a calf than to two men.

Laboratory colonization: A vigorous colony of *A. tessellatus* has been established at the National Institute of Virology, Pune.

Larval Ecology

In Pattukottai, the few larvae found were all in rice fields or channels, either in sun or shade, but in Malaysia, according to Reid (1968) they are found in dirty stagnant water under shade. In Maldives, the species was found breeding mainly in wells and step wells (Iyengar *et al.* 1953).

Relation to Disease

A. tessellatus is not a vector in the mainland of India, but should remain suspect. In Lakshadweep Islands, from where malaria had disappeared it had reappeared in 1972. Roy *et al.* 1974 and Roy *et al.* (1978) made further studies and concluded that the control of *A. tessellatus* again had brought down the disease in Minicoy Island where this was the only species found. Therefore, it is suspected to be the vector based on epidemiological grounds.

In Maldives where *A. tessellatus* and *A. subpictus* occurred, Iyengar *et al.* (1953) found that out of 22 specimens dissected one had heavy sporozoite infection. Covell (1944) has reported two gland and four gut infections among 160 specimens dissected earlier in Maldives. *A. tessellatus* is regarded as of some importance as a malaria vector in parts of Indonesia, particularly in West-Irian.

In Maldives, Iyengar (1952) considered it as a secondary vector of bancroftian filariasis, the main vector being *Cx. fatigans*.

Anopheles aconitus Donitz, 1902

Type locality: Kajoe-Tanam, Sumatra, Willem Island, Java, Indonesia.

Type: Zoologisches Museum, Humboldt University, Berlin.

Taxonomy: *A. brahmacharii* Christophers, 1912, first described from India, is now regarded as a synonym of *A. aconitus* because of the priority of the latter name.

Distribution: Throughout the Oriental region, from India to Indonesia and 'Indochina' and south to Sri Lanka. Absent in Taiwan and the Philippines.

In India: In all eastern and southern zones. Occurs in the Andamans but not in Lakshadweep. Not recorded from the western zones. It has been recorded from Karnataka and Kerala but not from Maharashtra, Gujarat, Rajasthan, Punjab, Haryana and Kashmir.

Distinguishing characters: Adult is of medium size. Readily recognised from its close relative *A. varuna* by a combination of the following characters: wing like a *minimus* but pale areas more extensive and a definite fringe spot on vein-6; female palpi with two broad apical pale bands but the intervening dark band is narrow; the female proboscis is fully flavescent in the distal half or 1/3rd both above and below. Larvae easily distinguished from *A. varuna* by the hair number-0 not arising on targal plate. For other characters please see Key.

Prevalence and Adult Bionomics

Though not so common as *A. minimus* or *A. fluviatilis* the species has been collected in houses and cattlesheds in India. However, most of the observations on bionomics have been made outside India. Christophers (1933) has given a few references of occurrence of this species in houses and cattlesheds. In Malaysia and Indonesia it is very commonly found, and is perhaps the most predominant species collected in houses.

Resting, feeding and biting habits: Very few observations have been recorded in India. It is predominantly a cattle feeder but Walch (1932), Yofe and Fox (1946) and Wharton (1951) have found that in south east Asian countries it bites man to some extent. In Indonesia where it is a malaria vector of some importance it occurs in houses. Wharton (1951), however, found that many more specimens were collected in cattle sheds than in houses.

The seasons of prevalence in India are not known. Russell and Ramachandra Rao (1940a) who collected only 72 adults in a two years study, found that it occurs between January and March i.e. a comparatively dry season, indicating that it was perhaps breeding in tanks or wells,the only two types of breeding places available in that season.

In Burma,the seasonal prevalence seems to be during the dry season with a peak in November and December. It is predominantly a cattle feeder, but will attack man if cattle are not available. Khin-Maung-Kyi (1971) states that it is usually an early biter. The invasion into the houses and cattle sheds starts as early as 18.00 hours and ends by 01.00 hours with a peak at 00.30 hours. Though adult specimens are found in houses, in some areas the species has been found outdoors in bushes. According to Venhuis (1942) many adult specimens can be collected along the banks of certain streams in Java.

Dispersal: Observations in other countries such as the Philippines and Indonesia, suggest that it disperses effectively upto a distance of a kilometre (Galvao, 1948). Russell and Santiago (1934) indicated a dispersal to shorter distance.

There is very little information on other aspects of adult bionomics applicable to India.

Larval Ecology

Christophers (1933) has referred to occurrence in clean tanks with grassy edges in Calcutta and also in ponds and storm water drains. Very similar habitats have been

found in Assam. Strickland and Choudhury (1927) had found that this species was breeding in river-bed pools. The species also breeds in rice fields particularly when the rice lodges at the end of the season (Senior White *et al.* 1943). Similarly, Sen (1948) found in Bengal that the greatest breeding occurred after the rice was at least 1.5 feet tall (45 cms). Sen (1941) also had shown that *A. aconitus* breed in waters containing a variety of algal growths and aquatic plants. *A. aconitus* showed a special perdilection for waters containing water hyacinth. It may be noted that Covell and Pritam Singh (1942) had also noted the association of this species with pools in rice fields around the Chilka Lake. In Malaysia and Indonesia it is a swamp breeder (Nair, 1947). Temperatures between 25 to 29°C have been found to be favourable for the distribution of this species (Senior White, 1946b).

Relation to Disease

In India *A. aconitus* has been regarded as a secondary vector next to *A. annularis,* in the Orissa coastal plains (Senior White *et al.* 1943). They found three gut and two gland positives out of 951 dissected. The species has also been occasionally found infected in Orissa, Assam and East Central India, by Senior White and Adhikari (1939), Anderson and Viswanathan (1941), Viswanathan *et al.* (1941) and Das (1943). Wattal (1961) has provided a fairly extensive list of the thousands of dissections made in many localities of India and the few positives recorded. Quite obviously the species is not a vector of any significance except locally in east central India, Orissa and Assam.

A. aconitus is a speices of some importance in Java, Sumatra, "Indochina" and Malaysia. In all cases it is comparatively a weak vector. In mid-Java, the two main malaria vector are *A.sundaicus* breeding in brackish coastal waters, and *A. aconitus* breeding in rice fields (Sundararaman *et al.* 1957). Subsidiary vectors are *A. maculatus* and *A.subpictus. A. aconitus* is the most widespread vector in Java breeding in rice fields over most of the island.

It has already become resistant to organochlorine pesticides.

No information is available regarding its role in *filariasis.*

Anopheles fluviatilis James, 1902

Type locality: Duars (Bengal), Nagpur (Maharashtra) and Jeypore Hill Tracts (Orissa).

Type: Unknown.

Synonyms: *listonii* Liston, 1901, Elichpur, now in Maharashtra.

leptomeres Theobald, 1903, Lahore, Pakistan.

arabica Christophers and Khazan Chand, 1915, Muscat, Oman.

For a long time, till Edwards (1932) decided to resurrect the name *A. fluviatilis,* it was generally known in India as *A. listonii* Liston 1901, which is now regarded as a synonym There has been much confusion regarding the identity of this and the related species, *A. minimus,* both of which were also incorrectly considered as synonyms of *A. funestus* of Africa. *A. funestus* Giles and its varieties in Africa are

known to be quite distinct. Now there are four recognised species of the *funestus* group in India viz. *A. fluviatilis, A. varuna, A. minimus* and *A. aconitus.* (For details see Edwards (1932) and Christophers, 1924 and 1933). See Addendum under *A. minimus*).

A. fluviatilis belong to the groups *Myzomyia* of Christophers (*Myzomyia* series of Reid) of the subgenus *Cellia.* The other members of this series occurring in India are *A. minimus, A. varuna, A. aconitus, A. culicifacies, A. jeporiensis* and its var. *candidiensis* and *A. majidi.* One other species, *A. dthali,* seems to occur only in Kashmir. *A. sergentii* originally from India, is not known to occur within the present day boundaries of India. It is found in the north western parts of Pakistan. Three other species of this group are reported from Southeast Asia, viz. *A. pampanai* known from Kampuchea, Thailand and Burma, *A. mangyanus* known from the Philippines and also reported from Nepal (Wattal 1963; Brydon *et al.* 1961) for which confirmation is needed and *A. filipinae* known from the Philippines with records from Nepal (Pradhan and Brydon, 1960).

Distinguishing characters: The adult *A. fluviatilis* is a small to medium sized mosquito. It is distinguished mainly by: presence of fringe spots on wing on all but vein 6; the basal quarter of costa with no pale areas or no pale scales even; vein 3 extensively pale; leaflets of phallosome 3 on each side sometimes with an additional small spicule. Larvae can be distinguished by the large tergal plate; hair—O arising a little behind the posterior border of tergal plate. Not easily distinguishable from larvae of *A. minimus.*

Distribution: Extensively distributed in the Oriental region and parts of the West Asian subregion, Afghanistan, Pakistan, Kazakh, USSR, Iraq, Iran, East and South Arabia, Oman and Behrain. In the Oriental region it occurs in India, Afghanistan, Pakistan, Nepal, Bangladesh, Burma, Thailand, "Indochina" and South China. According to Stone and Delfinado (1973) and Knight and Stone (1977) it occurs in Taiwan, but according to Reid (1968), it is absent from Taiwan and the Philippines. Christophers records a specimen from Sri Lanka but it is doubtful.

In India, it occurs in all mainland zones except perhaps the extreme south eastern section of Tamil Nadu. No records in the Andamans and Lakshadweep.

Prevalence

A. fluviatilis is essentially a species of the hills and foot-hills, occurring from sea level to altitudes upto 2,500 metres above M.S.L. It was incriminated by the author as a vector of malaria at Vikhroli at a few metres above sea level hardly 10 kms from the main Bombay Island and now part of the Greater Bombay. It is prevalent in Kashmir at altitudes of about 1,800 metres (Nair, 1973). Christophers (1933) states that he had collected specimens in Murree (Pakistan) at about 2.270 metres. It occurs in the high lands of Nepal as for example in Gum valley (altitude: 1,060 to 3,340 m).

There is strong reason to suspect that there are two biological races, one prevalent in the hills and foothills, characterised by high anthropophily, small densities, and high infection rates and another in the plains, as in the Deccan plateau, with

high zoophily, high densities and low infection rates. No morphological differences have so far been noticed. For instance, in the Nira Canals Zone (in the Deccan plateau of Maharashtra) it constituted more than a quarter of all *Anopheles* adults collected (55,095 out of 197,236 of all species), while in the Western Ghats of North Kanara District, only 2,144 adults were collected (5 per cent) out of a total of 42,960 of all species, and in Thane District 792 adults (19 per cent) out of 4,059 of all species collected (Viswanathan, 1950). In the Malnad areas of Karnataka (Hassan District) only 136 (0.9 per cent) adults were collected out of 14,773 adults of all species (Brooke Worth, 1953). The present author has himself made careful comparative studies of the species in all stages between the forms existing in two areas, i.e. Western Ghats and the Deccan, and has not found any morphological differences (unpublished). It has to be seen whether modern advances in cyto-taxonomy will be able to unravel this problem. Chowdaiah and Seetharam (1975) have so far examined the salivary gland chromosomes of only the form which occurs in the zone of high anthropophily. The probable existence of two geographical races has been suggested by several authors, such as Senior White (1946b), Viswanathan (1950), Brooke Worth (1952), Bhombore *et al.* (1954), and others.

Within the range of climatic conditions occurring in Peninsular India, it can be present throughout the year. However, the seasons of prevalence of this species is largely determined by the amount of rainfall and terrain.

A. fluviatilis occurs throughout the year in the plains and foothills. The seasonal prevalences depend more on the availability of breeding places than on atmospheric conditions. In North Kanara, where the species has been studied intensely, there were three distinct zones: (1) a western coastal area with very heavy rainfall and little breeding of *A. fluviatilis;* (2) a middle mountainous zone of heavy rainfall and thick forests, deep valleys and more streams than rice fields; and (3) an eastern plataeu zone of gently undulating land with moderate rainfall and abundant rice fields (Viswanathan, 1950). In the second of these zones *A. fluviatilis* became extremely scarce during the rainy season because of the flushing effect in the streams and cannels. A few specimens which occurred were those which bred in terraced rice fields or wells. In fact the rainy season was the healthiest part of the year with very little transmission of malaria. Intense breeding of *A. fluviatilis* in the perennial streams commenced only after the end of monsoon in October/November and reached its peak by about April and May. This was the season of intense malaria transmission. In the third zone, *A. fluviatilis* was most prevalent during the monsoon from June to October. The rainfall was not high enough for the flushing effect and there was also extensive breeding in rice fields. The fields and channels dry up fast after the monsoon and the species become scarce from January to June. More or less similar conditions occur in other parts of India also, i.e. the period of heavy rain is not suitable for the species. The season of maximum prevalence of this species has to be determined for each place carefully.

Adult Bionomics

Resting habits: Adults of the species are usually found, during day time, resting

in human dwellings and to a lesser extent in cattle sheds. There is, however, reason to believe that a considerable proportion of the population at any given time rest outdoors also. The habit of resting oudoors has been known, for a long time, in many parts of India, but there seem to be exceptions as in Hassan District (Achuthan *et al.* 1956). Among the studies made subsequent to Christophers (1933), a few important ones may be mentioned.

In North Kanara in Western Ghats, Jaswant Singh and Jacob (1944) found the species commonly resting in houses. Viswanathan and Ramachandra Rao (1943), working in the same area, had no difficulty in collecting adults inside houses during daytime but when they made further critical studies on nocturnal movements (Viswanathan *et al.* (1944) they found that a considerable proportion of the adults at a given time were resting outdoors. They estimated that about 60 per cent rested outdoors during daytime during the warmer months and about 40 per cent during the cooler months. Senior White (1946) and his colleagues have made extensive studies on adults of *A. fluviatilis* in east central India. They also considered that a sizeable fraction of the adult populations was resting outdoors during daytime in the Satpura ranges. Examination of the abdominal conditions indicated a dangerously high proportion of *fluviatilis* and *culicifacies* resting outdoors. But in Singhbhum hills only about five per cent of the specimens collected were resting outdoors.

It should be noted that collections of adult anophelines in outdoor places is very difficult unless special techniques are used. The few numbers collected do not fully reveal the actual numbers or proportions resting outside the dwellings. In sylvan surroundings the possible resting places are numerous and far out number a few houses which occur in such localities. Adult mosquitoes tend to become concentrated in the houses or cattle sheds where they can be captured with ease, but they are scattered outdoors over wide areas and in many types of shelters such as stream banks, vegetation, tree buttresses, holes in the ground, etc. It would be impossible to compare the numbers collected in houses with those actually collected outdoors. The effort required is disproportionately large in outdoor shelters. The fact that even a few specimens can be collected outdoors indicates that they can rest in such places, but the actual proportions resting outdoors and indoors would have to be deduced from critically conducted experiments rather than by direct comparison of numbers collected.

Gonotrophic conditions of mosquitoes collected indoors during daytime give some indication of the resting habits of the adults though they may not give accurate quantitative expressions. Particularly regarding *A. fluviatilis,* Viswanathan *et al.* (1944) made the following observations in regard to adult *A. fluviatilis*, catches made indoors, classified according to abdominal conditions:

(a) Unfed	...	15
(b) Freshly fed during the previous night	...	322
(c) Semi-gravid	...	128
(d) Fully gravid with a trace of blood but ready for oviposition	...	99
(e) Fully gravid with no trace of blood	...	11
(f) Miscellaneous conditions	...	1

If there was no outdoor resting at all the total of *'c'* and *'d'* should have been nearly equal to that of *'b'*, unless there was heavy mortality even on the first day of feeding, but it is known that *A. fluviatilis* in that area has a high longevity and the daily mortality would perhaps be less than 10 per cent. The proportion of *'c'* plus *'d'* indicates that many specimens which fed inside houses had left the indoor shelters. The females in class *'e'* were actually specimens which had missed laying eggs on a 48-hour cycle and had skipped a day. Further, 16 *A. fluviatilis* females were collected in outdoor shelters in a search of nearly 19 man-hours. All specimens had taken at least one blood meal and most of them were multiparous. During the same period only four specimens of this species were collected from houses in a total search of 34.5 man-hours. While one should not try to compare directly the numbers collected outdoors and indoors because of the very different types of efforts needed there is no doubt that a good proportion of the population does rest outdoors during daytime.

It is generally agreed that among the adult specimens found resting indoors, many more are found in human dwellings than in cattle sheds. However, Bhombore *et al.* (1954) collected 21 adults in houses, 69 in mixed dwellings and 247 in cattle sheds, quite in contrast with the observations made by others in North Kanara and Wynaad in the same range of hills where more adults were collected in houses than in cattlesheds. They also found that *A. fluviatilis* in the hilly western area of Mysore State (Hassan District) did not seem to rest outdoors to any extent in contrast with the observations of Senior White during 1941-46, Viswanathan and Ramachandra Rao (1943), Viswanathan *et al.* (1944), Covell and Harbhagwan (1939), Issaris *et al.* (1953) and others, in different parts of the country. Bhombore *et al.* made 'vigorous' searches extending over 5 months along banks of streams, under culverts, in clefts along the banks, and in paddy fields and did not find a single *A. fluviatilis* though members of other anopheline species were found. In window trap studies they found only one specimen of the species, but unfortunately they do not give the number of other species found in the traps.

However, the Mysore workers found many other differences also from the findings of the North Kanara workers in an area hardly 200 kms away. They found such differences also in almost all aspects of biology including anthropophily, time of biting, etc. It makes one feel that the two groups of workers were dealing with quite different biological races or strains. The Mysore workers themselves postulate that there may be different populations of a given species of anophelines in South India living within a few miles of each another, in this connection, the experience in the Deccan plateau where the species is mainly zoophilic and occurs in large numbers in cattlesheds should be borne in mind. Perhaps the Hassan population is similar to that of the Deccan.

Senior White *et al.* (1945) also noted that *A. fluviatilis* females remained in the hut where they had fed rarely more than one day. They found specimens in many types of outdoor shelters such as banks of nullahs, shade in vegetation, crevices under stones and also in terraced rice fields, under bridges, etc. and it appeared that the distribution of the adults had no relationship with the proximity of breeding

places. Issaris *et al.* (1953) studying the species in the Terai region of Uttar Pradesh also found *A. fluviatilis* resting in houses more than in cattlesheds.

However, in the Dhanbad area Sen, *et al.* (1960) found that *A. fluviatilis* was only second in abundance to *A. subpictus* and that it appeared to have changed its resting and feeding habits preferring cattle sheds as daytime shelters contrary to the earlier findings of G.R. Rao (1944).

Indirect evidence of the existence of outdoor resting in the Western Ghats comes also from the failure of the pyrethrum spraying as a means of malaria control. Even twice or thrice a week spraying did not have a marked effect on malaria transmission in North Kanara District (Viswanathan *et al.* 1944; Viswanathan 1950). This aspect will be discussed later when dealing with control.

Considerable outdoor resting was detected in Gujarat State. Shalaby (1971) dug eight artificial pit shelters at different locations in Panchmahals District. During a period of 10 months from October 1961 to September 1962 he collected 3,377 *A. culicifacies* adults and 176 *A. fluviatilis* adults. The highest captures were between December and April. Both males and females were found, the proportion being:—

	Males (per cent)	Females (per cent)
A. culicifacies	53	47
A. fluviatilis	23	77

The abdominal conditions of females were (per cent):

	Unfed	Fully fed	Half gravid	Fully gravid
A. culicifacies	55	20	21	4
A. fluviatilis	36	24	29	11

These observations definitely indicated that a considerable degree of outdoor resting took place in both species in all stages of the gonotrophic cycle, while freshly hatched ones predominated.

Direct collections of resting adults out of doors would be the best proof of such a habit. In Pune District, Ramachandra Rao and Rajagopalan (1957) found over a two-year period 199 adults inside dwellings, and 107 in outdoor shelters.

All indications are that a good proportion of *fluviatilis* adults rest outdoors during daytime,the proportion varying from place to place.

Nocturnal habits: Nursing *et al.* (1934) studied the nocturnal habits in Old Mysore State and found that as many *A. fluviatilis* entered their tent in the second half of the night as in the first. In North Kanara, Viswanathan and Ramachandra Rao (1943) studied the time of entry and time of feeding of *A. fluviatilis*. In night observations made in May and June 1943 when the total period of the night was only 10 hours, they found that hungry females entered human dwellings, mainly in the first part of the night, though some feeding continued even after midnight. Their study also showed that many gravid and semi-gravid individuals present in the earlier part of the night left the premises by midnight. *A. jeyporiensis* was also

collected in small numbers and appeared to have the same type of behaviour as *A.fluviatilis*.

Continuing further investigations, Viswanathan *et al.* (1944), by releasing marked specimens at various stages of gonotrophic cycle, determined that there was quite a significant degree of movement outdoors during the night leading to a substantial proportion not remaining in houses during daytime. They also noted in a series of critical studies in 30 nights, that 71 per cent entered the human habitations during the first quarter of the night, 19 per cent during the second quarter and small numbers later.

Observations were made on the behaviour of gravid females marked with printer's gold powders, released in a hut or a tent on four different occasions. Out of 285 fully gravid females released 15 were found to have returned for a blood meal after oviposition, even within a few hours after release and two on the subsequent night.

Jaswant Singh and Mohan (1951) made some very interesting observations in the foot-hills of Nilgiris. The period of maximum entry, viz. 64 per cent, was in the second and third quarter of the night. The numbers caught in each quarter of the night were (all year figures)

1st quarter	542
2nd quarter	884
3rd quarter	692
4th quarter	320
Total	**2,438**

Though 60 per cent entered before midnight ready for feeding, the feeding went on throughout the night. About 10 per cent of the unfed females re-entered into a hut on different occasions and were re-captured in the hut in the same night indicating that a considerable degree of outward as well as inward movement took place even during the night. Partially gravid females, when released, moved out of the hut into which they were released, though there was no physiological need for it. A good proportion of the females which had fed on the previous night and were released into a hut in the forenoon returned to the hut on the next day ready for feeding, indicating a gonotrophic cycle of 48 hours. The exact season of the study was not stated. A certain proportion took a double blood meal in the same night and a further proportion took the second blood meal on successive nights during the same gonotrophic cycle. This study is one of the most exhaustive ones on the nocturnal habits of *A. fluviatilis* and should be read in original. Senior White (1946*a*), in Hazaribagh ranges of east central India found that *A. fluviatilis* was rare in night catches except in October and November, the months of their greatest prevalence. There was higher prevalence before midnight. Bhombore *et al.* (1954 & 1956), Achuthan *et al.* (1947) and Brooke Worth (1953) showed that the time of entry in their area in Mysore State was throughout the night rather than in the earlier part, supporting the observations of Nursing *et al. (loc. cit.).*

Obviously, the time of entry for purpose of feeding is variable in different areas

and perhaps is determined by environmental factors or due to variations in habits of different strains. The most important finding however was that *A. fluviatilis* did enter man-made structures for taking blood meals, a habit which had such a great impact on malaria control by use of DDT indoor residual spray.

Host preferences: It is generally recognised that *A. fluviatilis* is predominantly an anthropophilic species. In Wynaad in Kerala, Covell and Harbhagwan (1939) made a series of preciptin tests on the species and found that 97 per cent of 1,681 stomach bloods tested had human blood. In North Kanara District Jaswant Singh and Jacob (1944) found 63.6 per cent to have human blood. In east central India Senior White (1947) found 56.8 per cent with human blood. Bhatia *et al.* (1957) detected a human blood index of 38.2 per cent in Rajasthan. In Thane District near Bombay City, Viswanathan (1950) has recorded an anthropophilic index of 84 per cent.

However, the anthropophilic index is low in several other parts of the country. For instance, in Assam Ramsay *et al.* (1936) found an index of only 3.8 per cent and Barber and Rice (1938) found an index of only 4.6 per cent in Pune, Maharashtra. Viswanathan (1950) has recorded that in the Deccan plateau the index is very low, 2.3 per cent in Pune District and 4.6 per cent in Bijapur District. Similar observations of low anthropophily have been reported in Uttar Pradesh Terai (1.8 and 1.4 per cent in 1928 and 1939 respectively (Sharma 1961 quoting annual reports of the M.I.I.). Issaris *et al.* (1953) noted that in the Terai area, *A. fluviatilis* was predominantly zoophilic. However, in the latter locality Ramakrishnan and Satyaprakash (1953) observed that from 1949 to 1952 the anthropophilic index had increased to 47 per cent. During the same period an infection rate of 1.6 per cent was also recorded by Srivastava and Chakrabarti (1952). The possible factors which brought about the change have been discussed by Ramakrishnan and Satyaprakash. While precipitin tests are a reliable tool in the study of malaria epidemiology, they are not themselves the final tools for determining the prediliction for hosts. However, there should be ecological reasons for this marked change in the feeding habits. Terai area was formerly densely forested and poorly inhabited though highly malarious. Much of the area has now been reclaimed and made suitable for agriculture. The area has now been colonized (Srivastava, 1950).

In a coalfield area of Bihar, Sen and Azeez (1963) found that all 97 stomach bloods examined contained bovine blood. The possibility of two biological races occurring in the country is further supported by these different kinds of observations

Longevity: Under laboratory conditions *A. fluviatilis* females were found to live upto 18 days at 20°C at any relative humidity. At 35°C and 100 per cent relative humidity they survived for 10 days and at 30°C and 60 to 80 per cent relative humidity they survived 17 to 18 days. Temperatures between 20°C and 30°C and relative humidity from 50 to 80 per cent provided optimum conditions for survival. These observations were made by Pal (1943) in Lahore.

Longevity under natural conditions, which is more important for epidemiological evaluation, has not been adequately determined. This species in many areas of the country shows very high sporozoite rates, sometimes as high as an average of 20 per

cent (Covell and Harbhagwan, 1939). In one limited locality, Viswanathan and Ramachandra Rao (1943) found in North Kanara, a sporozoite rate of 37.0 per cent. As the sporozoite positive adults should have lived at least for a period of 10 days, it is clear that the average longevity of *A. fluviatilis* in such areas would be quite high. In other areas such as the Deccan plateau where the sporozoite rates are much lower, the longevity is certainly much lower.

Some indirect studies have, however, been made by using Detinova's technique as re-described by Giles (1958). Achuthan and Sitaraman (1958) obtained the following data on the basis of 22 *A. fluviatilis* dissected in Mandya area, a district in the plains of Karnataka.

27 per cent	.. Nulliparous: less than 3 days
36 per cent	.. 1-parous.. 3 to 5 days
18 per cent	.. 2-parous.. 5 to 7 days
14 per cent	.. 3-parous.. 7 to 9 days
5 per cent	.. 4-parous.. 9 to 11 days

Noting that these dissections were made between August and October at a time when the gonotrophic cycle could be taken to be 3 days for first cycle and 2 days for subsequent cycles, at least 5 per cent had lived for 9-11 days.

More detailed studies were made by Sen and Azeez (1963) in the coal mines area of Dhanbad in Bihar. They dissected 171 *A. fluviatilis* by Polovodova technique in the cool months of December and January 1961, when the gonotrophic cycle was certainly longer (3 days), found

30 per cent	.. Nulliparous	22 per cent	.. 2-parous
47 per cent	.. 1-parous	1 per cent	.. 3-parous

They did not find any which were 4-parous. Using Achuthan and Sitaraman's formula they computed the calendar age. They showed that 23 per cent of *A. fluviatilis* females were at least 10 days old and one per cent over 13 days old and therefore in the potentially or in the epidemiologically dangerous age.

In both Mandya and Dhanbad, the survival of this species was without the selection pressure of insecticides because DDT spraying had been stopped in Mandya in 1955 and in Dhanbad in January 1961 about 11 months prior to the studies. In Iran (Eshghi, *et al.*, 1976) have found females surviving seven gonotrophic cycles in nature.

Flight and dispersal: There is no direct experimental evidence regarding the distances upto which *A. fluviatilis* adults can fly or disperse. Indirect observations on the location of the breeding places have, however, given some indications. The general impression is that *A. fluviatilis* adults do not fly far away from the source of feeding. It need not be stressed that various factors such as the type of environment, and the availability of suitable breeding places, will influence the range of flight and dispersal. Working in the wooded areas of Wynaad, Adisubramanian and Vedamanikkam (1943) found that the maximum distances from human habitations where larval breeding was found were about 1000 feet (300 metres). When the breeding was well controlled by oiling the breeding places upto that distance there

was a sharp drop in the number of adults in the houses. They obtained excellent results by just trimming the edges of perennial streams for a length of one mile. Venkat Rao and Philip (1947) found in Hazaribagh District in Jeypore hill tracts of east central India, that *A. fluviatilis, A. minimus* and *A. varuna* bred in significant numbers upto half a mile from the place where they fed. Achuthan *et al.* (1947) found maximum number of larvae upto 3 furlongs (approximately 600 metres) at 6 furlongs (approximately 1,200 metres) there were no larvae. While no specific and critical studies were made by the present author in North Kanara, he had found it easiest to obtain the larvae from breeding places very close to isolated clusters of human habitations. During attempts at control of the breeding of *A. fluviatilis* by anti-larval measures in selected villages in North Kanara, the author had found that it was necessary to go atleast to a distance of half a kilometre in large villages and hardly 200 metres in the case of small isolated clusters of huts. From these observations it is difficult to draw any precise conclusions regarding the actual flight range, but the breeding places were generally close to the houses. The flight or dispersal range, however, may vary from place to place. Perhaps there are different patterns of dispersal in different parts of the country depending upon environmental factors and the anthropophilic character of the species. Anyhow the flight and dispersal of *A.fluviatilis* appears to be of a much lower order than those of *A. culicifacies.*

Laboratory colonization: Mohan (1945, 1951) successfully for the first time, established a colony of *A. fluviatilis* collected from the Nilgiris area, in cages of 60x60x60 cms in the presence of a blue light provided by an ordinary five celled battery light which was covered by a blue cellophane paper. While the larvae could be reared successfully in infusions of hay with an addition of small quantities of litmus milk and dehydrated blood serum, there was a difficulty in the case of adults because the females were reluctant to feed on rabbits, guinea pigs or fowls. However, it was noticed that this difficulty was only with primi-para. If the colony cage was well stocked in the beginning to provide an adequate proportion of multi-parous females, the colony could be successfully maintained. The colony was maintained for several years.

Critical density: Under the condition in North Kanara, i.e., small numbers, high anthropophily and high infection rates, Viswanathan and Ramachandra Rao estimated the critical density of *A. fluviatilis* as 4 per 10 man hours. There is no doubt, however, that the critical density may vary substantially in different areas. Achuthan *et al.* (1947) estimated that in Hassan District, the critical density was much higher. In the Deccan plateau, the critical density of the species seemed to be the same as that of *A. culicifacies.*

Gonotrophic cycle: In North Kanara, Viswanathan *et al.* (1944) determined that *A. fluviatilis* had a normal gonotrophic cycle of 48 hours in warmer months and 72 hours in the cooler months. There is nothing to suggest that this could be very different in other parts of the country. In laboratory experiments (Senior White *et al., loc. cit.*) it was 48 to 96 hours when mosquitoes took only one blood meal for completion of ovarian development and 70 to 120 hours for oviposition. These data

are in agreement with field observations which indicate that in cooler months a minimum period of 3 to 4 days (72 to 96 hours) is needed for completion of the gonotrophic cycle. Jaswant Singh and Mohan (*loc. cit.*) have demonstrated that while maturation of the ova would be completed with only a single blood meal in a proportion of the females, many individuals require a double feed within one cycle.

Larval Ecology

A. fluviatilis larvae have a preference for breeding places with a perceptible flow of water, such as streams, field channels, ditches, etc. Occasionally they are also found in small pools by the side of streams, but even in such places there is generally a quick replacement of water (Puri 1931 and Christophers, 1933). In more recent studies in Wynaad, *A.fluviatilis* was found to breed normally in irrigation channels and streams with grassy edges. They also sometimes were breeding in shallow wells (Covell and Harbhagwan, 1939). Breeding in the shallow wells was found during the monsoon months when the streams and channels were flushed by heavy floods.

In North Kanara Ramachandra Rao (1945), basing his observations on the examination of 9,526 breeding places between August 1942 and December 1944, presented the data shown in Table 23a.

Table 23a. *A. fluviatilis* **breeding places in N. Kanara District**

Breeding Places	Times searched: times positive of any species	Total number of anopheline larvae of all species	Number of times *A. fluviatilis* larvae found	Percentage of times *A. fluviatilis* found	Total number of *A. fluviatilis* larvae found
Tanks	1,885	23,552	9	0.5	17
Rice fields (growing)	1,386	15,463	102	7.4	244
Rice fields (fallow)	797	9,679	42	5.3	98
Streams	1,931	19,367	258	13.4	815
Channels	1,685	15,335	235	14.0	865
Wells	906	8,318	75	8.3	142
Swamps	157	1,579	40	25.5	290
Areca garden trenches	212	1,614	4	1.9	9
Borrow pits	286	2,583	1	0.3	1
Miscellaneous	281	1,886	35	12.5	78
Total	9,526	99,356	801	8.4	2,557

Obviously borrow pits and tanks were the least attractive. Swamps were the most preferred, followed by streams and channels. He defined swamps as uncultivable fallow fields with flowing water and abundant vertical vegetation, but their number

was comparatively small. While these studies confirmed the importance of swamps, streams and channels as ecologically the most preferred places, he re-assessed the epidemiological importance of rice fields. Most of the rice fields, in which *A. fluviatilis* breeding was found, had a perceptible flow of water. The larvae also were more often found in the upper parts of the fields when they were terraced. Dense growths of rice plants and a steep bank provided good shade in addition to the flow.

In the extensive surveys in Western Ghats, Covell and Harbhagwan (1939) in Wynaad, Russell and Jacob (1942) in the Nilgiris and Jaswant Singh and Jacob (1944) in North Kanara, the status of rice fields as breeding places of the species had been considered but with varying findings. Covell and Harbhagwan stated that *A. fluviatilis* larvae were not found in rice fields, fallow or otherwise. Russell and Jacob found only 9 out of 299 *A. fluviatilis* larvae collected in the rice fields. Jaswant Singh and Jacob found the species breeding rather uncommonly in rice fields. Bhombore *et al.* (1954), in describing the bionomics of *A. fluviatilis* in the hilly districts of old Mysore State, found only 0.2 per cent of the total number of larvae in rice fields. Senior White and colleagues found *A. fluviatilis* breeding in rice fields in east central India, particularly in terraced paddy fields in the foothills. In Wynaad, Vedamanikkam (1952) found, over a 10 year period, that 56 per cent of *A. fluviatilis* larvae captured were in streams and 22 per cent in channels and 10 per cent in contour drains. The remaining 4 per cent were found in a variety of other places and only 0.3 per cent in rice fields. However, presence of a flow of water seemed to be the most significant factor favourable for the breeding of *A. fluviatilis.*

It is clear that in spite of some conflicting observtaions, *A. fluviatilis* breeds in rice fields. Ramachandra Rao assessed that rice fields, though not ecologically the ideal breeding habitat, may have nevertheless a very important part of building up *A. fluviatilis* adult densities because of the extensive surface areas of rice fields around villages compared to the total surface area of the less numerous streams and channels.

A few observations, hitherto unpublished, made by the present author in the North Kanara in 1945 may be briefly described here. First, the eggs of *A. fluviatilis*, when reared in water brought both from streams and rice fields, hatched and the larvae grew to maturity in the laboratory. Secondly, eggs of *A. fluviatilis* obtained from females made to oviposit in the laboratory, when placed in natural channels; with flowing water and rice fields, hatched normally and grew to maturity in the channels but the larvae in the rice fields without flow did not survive. The eggs were held in the natural breeding places in floating cloth bags. Thirdly, it was generally found that the actual spots where *A. fluviatilis* larvae were found in streams, channels or other places had some degree of the shade or growth of grass. It was surmised that though eggs could be deposited anywhere in the channels, the larvae which hatched would move into places with shade. Clean weeded channels never had *A.fluviatilis* larvae and even in jungle streams larvae were found only when there was overhanging shade from trees or bushes for most of the day time. Often larvae were found in the shallow margins of streams with hardly a millimetre depth

of water. In such places larvae should be looked for by making pools in the water edge. But such places also had good shade from overhanging trees. Finally, in order to test the attraction to shade, a few laboratory experiments were carried out. White enamel basins of a diameter of 14 inches (35 cms) were used (Fig.7.)

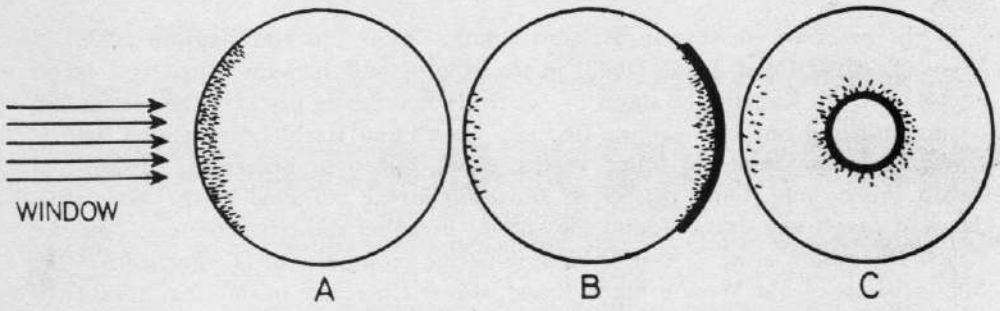


Fig. 7. Responses of *A. fluviatilis* larvae to light. Experimental. See Text.
A Basin with uniform white surface B Basin with a blackened edge.
C Basin with uniform white surface, but with a central cylinder with blackened edge.
The stippling indicates areas of concentration of larvae.

(a) When the basin was placed near a window, the water in the basin was well lighted, except for a slight shadow under the wall of the basin towards the window. When *A. fluviatilis* larvae were introduced, the majority of them actually moved towards the shadow and rested at the edge of the water. The edge of the water in the basins away from the window was well lighted. The larvae could perceive the shade from under the water surface and in trying to select the resting position, they actually appeared 'to move towards light'.

(b) When half of the basin was made black by fixing black paper, the larvae preferred to go to the black edge even when it was away from the window rather than to the natural but slight shade towards the window.

(c) When a cylinder of about 3" (7.5 cms) diameter, covered with a black surface (black paper), was placed in the centre of the basin, the larvae preferred to rest against the black edge of the cylinder rather than in the shade of the basin towards the window. The larvae appeared to perceive the darker surface and preferred it to the slight shade on the edge of the basin.

These preliminary experiments showed that *A. fluviatilis* larvae had a tendency to move towards shaded places because of negative phototaxy. This would explain the occurrence of *fluviatilis* larvae almost always in shaded places whether, under hedges, in grass-lined channels, or in deep shaded drains with or without vegetation, etc.

In another series of studies (unpublished) the effect of water temperature of certain types of breeding places on *A. fluviatilis* breeding was investigated. A small area near Sirsi town was selected. The area contained a tank of about 100 m diameter with an earthern bund. Below the bund were rice fields with well grown plants. Taking off from the centre of the bund and along the middle of the rice fields was a channel about one meter wide receiving direct supply of water from the tank through a sluice. The channel had grassy edges. Along the outer margins of the rice field area, there were two deep channels, one on either margin, not drawing water from the tanks or the rice field but getting plenty of seepage water through the banks on either side. They had practically no vegetation but had seepage water with a slow flow. Bordering the rice field area on either side were a few houses and cattle sheds. *A. fluviatilis* was breeding in the central channel and the two seepage channels and not in the rice fields or the tank. During early April 1945 a series of two-hourly observations were made for two consecutive days on the water temperature occurred in the shallow water of the rice fields varying from about 36° C at about 14-00 hours to 20° C at 06-00 hours. The tank had a more uniform range, because of the depth and mass of water, ranging between 28° C at 14-00 hours and 40° C at 06-00 hours. The central grassy channel had more or less the same temperature as the tank, the water of which it was drawing, at a place about 30 metres below the tank. A most uniform temperature was found in the two seepage channels with almost a constant temperature of 26° C throughout day and night. In fact the water of the drains felt, to the touch, warm at night and cool at day. With almost the same temperature conditions, the middle grassy channel was breeding *A. fluviatilis*, but not the tank. However, the most interesting finding was that at about 20-00 hours the temperature of all the breeding places was more or less the same, being about 26° C±1° C. If the gravid female had to exercise any choice in egg laying, temperature could not have been a very important factor because a little after dusk, at the time of major egg-laying, the temperature of all breeding places was similar. The statements often made by many workers that certain anophelines prefer 'cool' waters or 'warm' waters could be fallacious.

Relation to Disease

A. fluviatilis is among the most efficient vectors of malaria. It is a vector in all areas of India where it is prevalent. In certain areas, particularly hills and foothills, it is extraordinarily efficient, perhaps more efficient than any other Indian *Anopheles*. In other areas particularly in the plains, it can be classified only as a good vector at least as effective as *A. culicifacies*. It has also been found infected in Pakistan and Iran. Monoucheri *et al.* (1972) have stated that in the southern highlands of Iran, though *A. stephensi* is the main vector, *A. fluviatilis* and *A. dthali* are subsidiary vectors. Dissections have been carried out extensively in several areas. Christophers (1933) recorded Perry's detection in 1914 of a 4 per cent gland infection in Jeypore Hill States in east central India. Some of the high and low rates of infection found are shown in Table 23b.

Table 23b. High and low rates of infections found in *A. fluviatilis*

Locality	Authors	Sporozoite rate percentage	Total Infection rate percentage
High Rates:			
Kerala	Mathew (1939)	12.0	?
Wynaad, Kerala	Covell & Harbhagwan (1939)	13.0	20.2
North Kanara, Karnataka	Jaswant Singh and Jacob (1944)	7.1	11.0
North Kanara, Karnataka (One study)	Viswanathan & Ramachandra Rao (1943)	37.0	44.4
North Kanara Karnataka (Cumulative study)	Viswanathan (1950)*	4.3	7.0
Thane (Maharashtra)	Viswanathan (1950)* (*Average for four years)	3.1	4.0
South Kanara, Karnataka	Ramakrishnan *et al.* (1948)	8.7	?
Terai, U.P. 1949	Srivastava and Chakrabarti (1952)	11.1	?
Nilgiris Tamil Nadu	Russell & Jacob (1942)	10.1	17.3
Low Rates:			
Orissa	Senior White (1946)	2.6	?
Orissa & Bihar	Senior White (1947)	?	5.7
Nasik, Maharashtra	Viswanathan (1950)	0.1	0.1
Nira Canal Zone Maharashtra	Viswanathan (1950)		
Terai, U.P. (1951)	Srivastava and Chakrabarti (1952)	1.6	?
Terai, U.P. (1949-50)	Issaris *et al.* (1953)	0.1	?
Hazaribagh	Senior White (1941)	31/2167	?
Satpura, M.P.	Senior White & Adhikari (1940)	26/931	?
Assam	Chalam (1923)	12/315	?

It will be noticed that *A. fluviatilis* is a very powerful vector throughout the Western Ghats and Nilgiris. In east central India, Assam and Himalayan foot hills it is a vector of medium efficiency. Its lowest efficiency is in the plains such as the Deccan plateau. In the Western Ghats it is the main vector and occasionally shares the responsibility of transmission with *A. culicifacies*. In the Deccan it shares the malaria transmission with *A. culicifacies* and *A. stephensi* all playing about an equal role. In fact all three, *viz. A. culicifacies, A. fluviatilis* and *A. stephensi*, all with low infection rates and low anthropophily, are active as in the Deccan plateau. The critical density of all three species put together was about 5.0 per man-hour. In the east central India it shares the responsibility with species like *A. annularis, A. varuna, A. minimus* and *A. aconitus*, and to a lesser extent with *A. culicifacies*. In the U.P. Terai, rates as high as 11 per cent had been detected in 1949 (Srivastava and Chakrabarti, 1952). In 1951, however, the sporozoite rate was only 1.6 per cent. Previous studies in that area had shown that it had been a weak vector. In 1938 only two gut infections out of 1,817 specimens dissected had been found by the Malaria Institute of India (Sharma, 1961). Even previously, Clyde

(1931) had dissected 460 specimens in the U.P. Terai with negative results (Sharma, *loc.cit.*). Subsequent studies have shown rather low rates. Issaris found a sporozoite rate of only 0.12% in 1951.

The reasons for these changes are not well understood though the probability of increased contact between man and vector as a result of the colonization of this area, could have been a factor; so also the fluctuations in the anthropophilic index. District of Andhra Pradesh, (Abraham and Samuel, 1944), Bengal (Senior White recorded as of *A. minimus*. It is worth pointing out that *A. minimus* once considered to be the vector in the Terai is no longer so.

The other areas of India where natural infections have been found are Karnal now in Haryana and Udaipur District of Rajasthan (Bhatia *et al.* 1957), Nizamabad District of Andhra Pradesh, (Abraham and Samuel, 1944), Bengal (Senior White and Ghosh, 1946). *A. fluviatilis* has been incriminated as a local vector in Kashmir at altitudes between 500 to 1,900 m (Nair, 1973). Out of 56 dissected one gland positive was found at an altitude between 1,675 and 1,880 m.

It is of considerable importance in Nepal, particularly at the higher elevations.

A. fluviatilis has no importance in any other country to the east of India. No infection has been reported from Burma where the species occurs in a scattered manner. It plays an important role to the west of India particularly in Pakistan and Iran. Eshghi, *et al.* (1976) have recognised its importance in Iran (Sporozoite rate of 0.7 per cent).

Very few experimental studies have been made on laboratory infections and on the comparative susceptibility to infection with other species. One among the few studies is that by Mohan (1955). Comparing study of the relative susceptibility of *A. fluviatilis* and *A. stephensi* to infection with *P. falciparum* in laboratory conditions, he found that both the species were equally susceptible. Out of 252 *A. fluviatilis* dissected, 113 were positive either gut or gland or both infections, and out of 150 *A. stephensi* dissected, 81 were similarly positive when fed on the same carriers. The specimens for this experiment were obtained from laboratory colonies. This was the first infection experiment of this kind from specimens of a self-perpetuating four-year old laboratory colony of *A. fluviatilis*. The *A. stephensi* colony was, however, 17 years old.

There is no information on the ability of *A. fluviatilis* to transmit animal malaria or human filariasis.

Control

The control of *A. fluviatilis* as a means of controlling malaria had long been neglected. The recognition of the importance of the species as a major vector started only after the work of Mathew (1939) and Covell and Harbhagwan (1939) in Kerala State, though it was even previously known as a vector. With the establishment of the permanent Malaria Organization of Bombay State, with its first field of activities in North Kanara in 1942, serious efforts at control of *A. fluviatilis* transmitted malaria commenced. Simultaneously the Malaria Department of the Bengal-

Nagpur Railway under the leadership of Senior White also directed its attention to the species.

Based on the knowledge that the species preferred to breed in streams and channels, attention was directed towards the control of breeding. From 1942 to 1945 extensive studies were undertaken (Viswanathan, 1946, 1950). The methods adopted either singly or in combination were:

1. Clean-weeding of all channels and streams.
2. Use of paris green or copper cyanide, mixed with road dust, in all types of breeding places.
3. Pyrethrum space spraying.

Clean weeding of channels and streams was found to be quite practicable. At one time 8 miles (12.8 km) of water courses were kept completely clean weeded in a small town (Yellapur) and 5 to 6 miles (8.0 - 9.6 km) of channels kept completely free of weeds in each of another 6 villages by gangs of labourers. Not a single larvae of *A. fluviatilis* was found in the clean weeded channels even after very careful searches. While this method was effective in keeping the larvae away, the muddy banks of the channels gradually eroded, a feature not liked either by the farmers or the administrators.

Paris green or copper cyanide was found to be quite effective and economical so long as the materials were available. But it could not yield the degree of control which clean weeding provided. However, Paris green became absolutely essential when the role of rice fields was appreciated. As the breeding of *A. fluviatilis* in rice fields was concentrated in the vicinity of habitations, the method was quite practicable and the use of Paris green was also not beyond the ordinary economic capacity of the villages.

Pyrethrum space spraying applied once or twice a week, as in the case of *A. culicifacies* was found to be ineffective. The studies on adult bionomics indicated that because of the high anthropophily and exophily, many infected mosquitoes would escape being killed. Further investigations (Viswanathan *et al.*, 1944) on the degree of exophily and other related matters showed that if a four times a week spraying was undertaken, practical control of malaria was obtainable. A rational space spraying weekly schedule of two successive days of spraying with one and two days of sprayless intervals alternatively (2-1-2-2) would take care of much of the adult population. This programme was carried out in 1944-45 at the end of which considerable drops in spleen rates and a lowering of dispensary figures occurred. However, the appearance of DDT in 1944-1945 led to the complete termination of the above measures.

Senior White and colleagues also carried out many investigations of the control of *A. fluviatilis* in Bengal-Nagpur Railway. Their work with the use of pyrethrum space spraying showed similar results to those in North Kanara. However, Senior White (1945) stated that six times a week spraying was necessary to bring down *A. fluviatilis* transmitted malaria.

In an exhaustive and highly critical review of the utility of pyrethrum spraying in relation to the bionomics of anophelines, Senior White *et al.* (1945) made several observations of interest. Though they disagreed with some of the observations made by Viswanathan and Ramachandra Rao in North Kanara, they came to almost similar conclusions such as:

1. Pyrethrum spraying is more effective against *A. culicifacies* than against *A. fluviatilis* and related species.
2. They found very high percentage of survival, for over 12 days in the cold weather, *fluviatilis, minimus* and *jeyporiensis.*
3. Whilst *A. culicifacies* remains in human dwellings after feeding until digestion is nearly completed, the vectors of the *fluviatilis* groups leave the feeding point when the cycle is only half completed. The validity of deductions as to age drawn from wing stage is doubtful. In pyrethrum sprayed houses *A. fluviatilis* enter the dwelling mainly after midnight. This strongly suggested that pyrethrum spraying acts as a repellant to mosquitoes entering the rooms.
4. Against the *fluviatilis* group six days a week spraying is the most effective. In both *fluviatilis* and *varuna* there are definite indications of biological races.

This paper needs to be read by all as an example of an excellent review of mosquito bionomics even though one may not agree with all the conclusions.

DDT indoor residual spraying has been most effective against *A. fluviatilis.* The very first field experiments were made by Senior White (1945) and Viswanathan and Parikh (1946). Towards the end of 1944 and in 1945 Senior White showed the effectiveness of DDT in a few villages in Orissa. Viswanathan and Parikh (1946) did a much larger experiment in three sets of villages in North Kanara in 1945. By spraying DDT on the inside walls of houses, as a 5% solution in kerosine oil at a dosage of 56 mg/sq. ft. in two or three rounds, very dramatic reduction in density of *A. fluviatilis* was obtained. In 1946, DDT spraying was extended to two large districts, Dharwar and N. Kanara (total protected population about one million), with excellent results (Viswanathan and Ramachandra Rao, 1947, 1948 and 1949).

Similar results against *A. fluviatilis* were obtained by several other workers (Ramakrishnan *et al.*, 1948; Puri, 1947). The succeeding extensive use of DDT under N.M.C.P. and N.M.E.P. is well known.

The success of DDT against *A. fluviatilis*, in spite of its outdoor resting habits, may be attributed to its predominantly indoor biting habits and high susceptibility.

Effect of insecticides: The effect of DDT spraying on *fluviatilis* populations has been very marked in vast areas of peninsular India. Populations of the species seem to have undergone considerable diminution. First noted by Vedamanikkam (1952), there was a conspicuous reduction in the prevalence, almost to nil, indicating that the species was in fact becoming extinct in some areas.

The effect of contact with insecticides on the bionomics of mosquitoes which survived, is not well understood. Several papers have been published on the excito-repellant action which drives the mosquitoes out of the sprayed surfaces, but the subsequent behaviour is not well studied. Mohan (1955) carried out several experiments with laboratory bred colonies of *A. fluviatilis* and *A. stephensi.* Specimens

after exposure to sub-lethal doses of DDT in different stages of gonotrophic cycle were used. A single sub-lethal contact did not affect the development of eggs and there was close correlation between fertilization and maturation of ovaries irrespective of exposure to DDT. However, exposure to DDT had some effect on the females; eggs were laid at random though suitable water for oviposition was available. His findings are somewhat different from those of Pal *et al.* (1952) who found that there was a definite lowering of oviposition in *A. stephensi* which had come in contact with DDT.

A. fluviatilis has not yet become resistant to any synthetic insecticide, except for some unconfirmed reports in isolated localities like Pandharpur (Maharashtra) and Bijapur and Sakleshpur (Karnataka).

Anopheles minimus Theobald, 1901

Type locality: Pokfulam, Hong Kong.

Type: Location unknown. Christophers (1933) has stated that the type specimen was noted by Theobald as in Ree's collection. Harrison (1980) has now re-examined the taxonomy of the entire *minimus* group of Myzomyias. His review should be consulted for information on types and their locations.

Taxonomy: For some time the species was being named *A. christophersi* (Theobald), until Edwards (1915) established it as a synonym of *A. minimus*. Identifications of *A. fluviatilis, A. aconitus* and *A. minimus* prior to that date cannot be relied upon. There are several synonyms of *A. minimus* but only one valid subspecies, *A. minimus flavirostris*, of the Philippines, is known.

Distinguishing characters: The adult is a small to medium sized mosquito. It can be distinguished from close relatives by a combination of the following characters: female palpi with two broad apical pale bands separated by a short dark area; wing without a fringe spot on vein-6; inner quarter costa with one pale interruption or at least a few pale scales; female proboscis if flavescent only as a small patch on the ventral side of apical half. For larva see general key. It is difficult to distinguish it from larva *A. fluviatilis*.

Distribution: Very widely distributed in the Oriental region. India, Nepal, Sri Lanka, Bangladesh, Burma, Thailand, 'Indochina', Indonesia, Malaysia, South China including Hong Kong, Taiwan and the Ryukyu Islands. The species, occurs in Malaysia and Indonesia in scattered localities. The forms in the Philippines, Borneo, Java and Sumatra belong to the subspecies *flavirostris* (type locality: Luzon).

In India: Widely prevalent in the eastern, northern and east central India. Also recorded in scattered places in Andhra Pradesh, Tamil Nadu, Kerala and Karnataka. Not recorded from western and north-western India, *i.e.,* Maharashtra, Gujarat, Rajasthan, Punjab, Haryana, Kashmir and Delhi.

The records from southern India need re-examination and confirmation. Puri (1948) has recorded a specimen from Tanjavur District. Russell and Jacob (1944) and Russell and Ramachandra Rao (1940) did not find it in Tamil Nadu though

extensive collections were made for several years. Also there seems to have been a diminution of its population in recent years.

Prevalence

A. minimus is characteristically a species of the hills and foothills, but is also found in the low lying Brahmaputra Valley in Assam and Bangladesh and in the deltaic areas of the Bengal, even at low altitudes. It has been recorded upto altitudes of about 1,600 m (Shillong).

It has been a very common mosquito in the past. In 1937, Gilroy (1939) found that in the tea gardens of Darjeeling District *A. minimus* adults numbered 842 out of 17,849 of all species collected. It was a common species when Muirhead Thompson made his studies on the ecology of the species in Assam between 1938 and 1942.

There is evidence that in recent years the distribution of *A. minimus* has undergone a great change. It seems to have either disappeared or has become scarce in many of its old areas of prevalence. Ramachandra Roa *et al.* (1973), in their survey of the Himalayan regions, and also Varma and Mahadevan (1970), in their survey of eastern Himalayan foothills including Darjeeling, Kurseong, Kalimpong and Bhutan foothills during July to December 1966, did not find *A. minimus* in any of the localities visited. Only one specimen was collected by Rajagopal (1976) in Burnihat, Assam, where it had long been recognized as the main vector. Recent information indicates that in Nepal also it has disappeared. In 1970, workers of the NICD (NICD Annual Report, 1970) in their extensive studies in the Burnihat region of 'Assam' (Meghalaya) did not find a single specimen of *A. minimus* either in indoor resting collections or in collections biting on man or cattle. In Tirap District of Arunachal Pradesh also, Sen *et al.* (1973) did not find any specimens of *A. minimus*. As early as 1958, Sharma had already noted a virtual elimination of *A. minimus* in the areas where DDT had been sprayed. From information received through the courtesy of the Director, N.M.E.P., it seems that this great diminution of *A. minimus* population has continued to persist even today: 1979.

However, Ismail *et al.* (1974) in Thailand did not find difficulty in obtaining large numbers biting man, Ismail *et al.* (1978) found the species abundant in subsequent years. In Burma, Khin-Maung-Kyi (1971) makes no mention of the scarcity of the species; in fact this species was still regarded as an important vector. It is highly susceptible to DDT in both countries, but DDT does not seem to have affected its prevalence. A great diminution has been noted in Yunnan Province of China. Why the species has undergone such a change in India, Nepal and S. China only is a matter of interest. Intensified studies on night biting seems to be necessary.

Adult Bionomics

Resting places: It has long been recognised as a species easily collected in houses and cattle sheds during daytime. There are many early references to such a habit by Christophers (1933). Muirhead Thomson (1938-1942), who made a special study of the species, stated that *A. minimus* was a thoroughly domestic mosquito resting and

feeding entirely in houses; but later (1951) he expressed some doubt as he had not carried out any window-trap studies. Outdoor shelters were not specially looked for by most workers. Macan (1949), on the other hand, found in Burma that large proportions of adults left the houses after feeding and sought outdoor shelters. He found that in the forested areas in the Kabaw Valley *A. minimus* was rare in houses, but in villages in open cultivated land it was common inside houses. *A. minimus flavirostris* is very well known to be predominantly an outdoor rester (Russell, 1931) in the Philippines.

Though Muirhead Thomson (1941a) did not make any collections outdoors, he considered (1951) the proportions of the freshly fed and the gravid specimens inside houses during daytime as an indication of the degree of outdoor resting. In Assam, he found that the proportion of *A. minimus* freshly fed and gravid was 65 and 35 which indicated that a great deal of the time of the adults was spent outdoors. Senior white, Ghosh and Rao (1945) had actually collected *A. minimus* adults from outdoor shelters along streams in Jeypore hill tracts. Unlike *A. fluviatilis, A. minimus* adults do not usually rest in the lower half of the walls inside houses.

From recent reports from China (Chinese Medical Journal 1976, July, 257-263), *A. minimus* used to be the most common species (71.6 per cent of all anophelines) collected in houses during the years 1958-59 and was the only anopheline infected (sporozoite rate 0.4 per cent). However during the surveillance in 1972-75 only 26 *A. minimus* adults were collected in a total of 4,362 of all species. This indicated that in the Yunnan Province of China also there has been a great reduction in the population of the species as a result of control operations.

Biting, feeding and resting: The behaviour of adults of *A. minimus* received considerable attention from Muirhead Thomson in his classic studies (1938 to 1942). The gonotrophic cycle during the hot damp monsoon months was 48 hours, oviposition taking place the second night after feeding. However, in cold weather the gonotrophic cycle could take even 4 to 6 days. The behaviour of marked mosquitoes showed that there was a considerable degree of daily turnover in houses. There was strong attraction to light at dusk and most mosquitoes left the shelters at that time. There was no such reaction at dawn. About 90 per cent of blood feeding took place after midnight and there was complete absence of blood-fed adults in the earlier part of the night. *A. minimus* females returned after oviposition to take a blood meal the same night. There was no indication of hibernation in the cold weather. Senior White and V.V. Rao (1944) showed that most of the specimens collected indoors had fresh blood, indicating that females stay on in the places of feeding at least for one day, but they leave the houses for other shelters for the completion of the gonotrophic cycle.

Temperature: Various degrees of atmospheric humidity did not affect the reactions of *A. minimus* adults but there was strong avoidance of higher temperatures i.e., 25°C by hungry females and 30°C by blood-fed ones.

Nocturnal movements: Very little work has been done on nocturnal movements of *A. minimus* unlike studies on *A. culicifacies* and *A. fluviatilis*. Krishnaswami (1952) found that in Darjeeling District females of *A. minimus* entered human

dwelling mainly before midnight but maximum feeding took place after midnight and they stayed inside the dwellings, long enough to pick up a lethal dose of DDT. Apparently quite a number of fully fed specimens stayed on after feeding, indicating the absence of any excito-repellant effect. His data are reproduced below.

Hours		Not fed.	Partially fed.	Fully fed.	With partially digested blood.
15.00	..	20	1	2	20
21.00	..	7	0	2	31
23.00	..	24	2	8	7
01.00	..	14	6	33	11
03.00	..	8	5	48	5
05.00	..	4	5	41	3
07.00	..	0	4	24	5

In the Arakan, Burma, Macan (1949) found that the species entered houses by about 21.00 hours and the peak entry was reached at midnight though most of the feeding took place after midnight. Fox (1049), quoted by Khin-Maung-Kyi (1971), also working in the Mandalay region had made similar observations.

Khin-Maung-Kyi (1971) stated that after the countrywide use of DDT, the biting time varied from locality to locality and from season to season. In the Mandala region, the biting occurred in the first and second quaters of the night. In certain other areas, biting occurred in every quarter of the night.

There is some evidence that *A. minimus* female mosquitoes, after oviposition, can return for a blood meal on the same night (Muirhead Thomson, 1951).

Ribbands (1946) had found that in the *funestus* group of mosquitoes in Africa, the entry into houses was greatly influenced by the phases of the moon.

For many years in Thailand also *A. minimus* was believed to be the main vector; but now *A. minimus* continues to occur. Ismail *et al.* (1974) carried out detailed investigations on the behaviour of both *A. minimus* and *A. balabacensis* in a rural area (Pitesanuloke Province in Northern Thailand). *A. minimus* appeared in high density during dry season from November to February. It attempted to bite man more frequently outdoors than indoors during the dry period and the biting densities outdoors were usually 3 to 4 times higher than those indoors. During that season the species was found to bite throughout the night (both outdoors and indoors) with a high density during the first part with a gradual fall till 06.00 hours. However, during the wet season it was comparatively a late biter. Out of 3,591 females dissected 2,165 were parous giving an average parous rate of nearly 61 per cent. *A. minimus* was reluctant to enter experimental huts unlike *A. balabacensis.*

In a more recent study, Ismail *et al.* (1978), working in the northern part of Thailand, found *A. balabacensis* to be the main vector, followed by *A. minimus* which was still persisting in good numbers. In night biting collections 4.4 to 5.8 times more specimens of *A. minimus* were collected outdoors than indoors, with a peak biting time between 18.00 and 19.00 hours. In wet season, however the biting

activity exhibited a plateau between 21.00 and 03.00 hours. The gonotrophic cycle was 2 days in wet season and 3 days in dry season. From a study of the parous rates they concluded a life expectancy of 17.2 days before DDT and 4.6 to 7.1 days after DDT. So far no resistance to the insecticide had been found.

Previously in Thailand *A. minimus* was regarded as both endophagic and endophilic (Sambasivan *et al.*, 1953, unpublished, quoted by Ismail *et al.*, 1974). In other areas of its geopgraphical distribution also the same behaviour had been reported. This situation has changed considerably during the recent years when *A. balabacensis* has become the chief vector and *A. minimus* has become a secondary vector. Ismail *et al.* (1974) found in Thailand that *A. minimus* is now predominantly an outdoor biter and outdoor rester. In view of these observations, they postulate the possibility of the existence of two biological variants of *A. minimus*, the domestic race becoming scarce with DDT house spraying over many years and the exophilic variety occupying the forest fringe in the foothills, where conditions are suitable for its outdoor biting and outdoor resting behaviour. (see for further details under *A. balabacensis*).

It has to be seen whether such change has occurred in India, to a greater degree, to explain the absence in houses. Intense studies on biting collections at night are urgently needed.

Flight and dispersal: No critical observations are available on the flight range and dispersal of *A. minimus* adults. Earlier observations, such as by Harrison and Ramsay (1933) quoted by Muirhead Thomson that the flight range was not less than a 1000 yards (930m) was based on the location of the breeding places. In the Philippines, the subspecies *flavirostris* may have a flight range of over 2 kms (Russell and Santiago, 1934a). Galvao (1948) has recorded migrations of over 12 kms.

Muirhead Thomson's (1941b) studies showed that in Assam the movements of *A. minimus* adults took place both by night and day and that the day movement was mainly at the time of dusk.

Host preferences: *A. minimus* prefers human blood. Not only is this observation drawn from the higher numbers found resting in houses as compared to cattle sheds, but also from precipitin tests. Ramsay and Macdonald (1936), working in 'Indochina', found that 533 out of 562 stomach bloods examined were of human origin; and Senior White *et al.* (1945) in Jeypore hill tracts found 122 out of 134 (91 per cent). *A. minimus* is among the most anthropophilic of Oriental anophelines. Observations in other countries also, particularly China and Hong Kong, have shown very high anthropophilic indices ranging from 50 to 93 per cent. However, the sepcies is not averse to feeding on cattle blood when the need arises.

Gonotrophic cycle: Like most other species of anophelines in India, *A. minimus* also has a normal gonotrophic cycle of 48 hours (Muirhead Thomson 1941) but in cold weather it may take 3 to 4 days. Senior White *et al.* (1945) noted a little longer cycle.

Studies by Rice and Mohan (1936) on *A. minimus* in Assam during the winter months brought out the interesting fact that the species continued to breed and there was a constant supply of adults throughout the cold weather and transmission

was also occurring throughout. They found oocyst and sporozoite rates as follows:

	Oocyst rate per cent	Sporozoite rate per cent.
December . .	4.8	1.8
January . .	2.0	0.3
February . . .	2.1	0.0
March . .	8.6	2.6

They found no evidence, on the basis of studies of wing grades, ovarian stages and gut contents from December to March, of any hibernation, overwintering or gonotrophic dissociation. As with all the other anophelines studied in India, there is no real hibernation in *A. minimus*. (Muirhead Thomson, 1941).

Longevity: Undoubtedly, *A. minimus* in the regions where it is an efficient vector is long lived compared to many other anophelines. Sporozoite rates as high as 9 to 10 per cent cannot occur unless large proportions live longer than 10 days. Direct observations are very few, but females reared in cages have been reported to live 40 per cent for one week, 23 per cent 1 to 2 weeks, and 4 per cent 3 to 4 weeks. Muirhead Thomson pointed out that longevity in nature increased more in the humid parts of the year.

Colonization: *A. minimus* is generally difficult to colonize but by using forced mating techniques, Wilkinson *et al.* (1974) established a colony from progeny of wild caught females. Females oviposited 3-15 days after mating and larval development took 26 days. Pupal stage lasted 2-3 days.

Larval Ecology: It is well known that habits and habitats of larvae of *A. minimus* are similar to those of *A. fluviatilis*, and that the larvae are generally found in streams, ditches, channels, tea garden drains, etc., which have a perceptible but slow flow of water. Sometimes they are also found in shallow *katcha* wells. The species has occasionally been found also in borrowpits, rice fields and seepage springs. Christophers (1933), quoting Ramsay stated that during the monsoon it breeds in clear grassy streams and drains with some shade and also in seepages, but in cold water it breeds in more permanent places such as rivers, streams, grassy tanks and swamps. However, Ramsay and Macdonald (1936) stated that streams with grassy margins exposed to sunlight or only under *partial* shading were preferred. They also noted that the species rarely bred in a shallow unshaded rice fields; but when the water was cooler, as and when supplied by a cool seepage spring, rice fields were also found to breed the species. During the monsoon the streams and channels become unsuitable because of their velocity and presumably also due to silt.

The duration of larval life of *A. minimus* was studied by Robbands (1949). The shortest period was 7 days. In the monsoon conditions, the life of eggs was 2 days, larvae 7 days, pupae 2 days, adults upto first egg laying, 3 days.

The larval ecology of *A. minimus* was studied by Muirhead Thomson (1938 to 1942) in a series of studies using refined experimental techniques. Those studies

were not only able to clear many points regarding the breeding habits of the species but served also as a model for several subsequent studies on other species of anophelines. The observations by Muirhead Thomson deserve to be quoted in some detail.

(a) Selection of the breeding places: It has been well known that in Assam breeding of *A. minimus* has been associated with the grassy margins of clear unpolluted running streams. Stagnant marshes and rice fields rarely breed this species. Though there is some random scattering of eggs in different types of breeding places, streams and channels are the most prolific sources of *A. minimus*. Muirhead Thomson examined these problems from two angles: (1) Are the eggs being deposited at random in many different types of breeding places, but the resulting larvae are able to survive only in streams, etc? or (2) Does the gravid female play any part in the selection, depositing the eggs only in the types of waters best suited for the larvae? Using a white enamelled basin or frying pan to skim the surface of the water over as large an area as possible, he was able to collect and count fair numbers of eggs. In a series of five observations made in a well with grassy margins, 67 *A. minimus* eggs were collected and 217 larvae of the species were also found. Similar numbers were recorded from other wells over a period of one month. Eggs of the other species found were of *A. aconitus*—14, *A. "hyrcanus"/barbirostris*—10, *A. vagus*—22 and *A. kochi*—13. This study indicated that *A. minimus* did lay eggs in waters of wells with grassy margins and the larvae could survive. However, in a few borrowpits which had been freshly dug close by, no *A. minimus* larvae were found over a period of one month, while larvae of *A. vagus, A. "hyrcanus", A. barbirostris, A. annularis* and *A. philippinensis* were found. Examination of the surface of water of the borrowpits yielded not a single egg of *A. minimus* while eggs of other species were plentiful. It seemed, therefore, that the female of *A. minimus* showed some preference for certain types of water and avoided others, but some haphazard distribution of eggs also took place.

In such experiments it is essential to find out the time of oviposition as precisely as possible for without such an information erroneous conclusions may be drawn regarding the factors responsible for the actual field observations. Muirhead Thomson made a few laboratory observations with gravid females and found that 69 per cent of eggs were laid in the first third. It was obvious that the factors prevalent during the earlier part of the night would be important.

(b) Effect of shade: It had long been observed that one of the most effective natural methods of control of *A. minimus* breeding was the shading of the streams with hedges of various plants (Ramsay and Macdonald, 1936).

The effect of shade may be due to many causes including the following:

1. Gravid females may be repelled by dense shade.
2. Dense shade might be unfavourable for the growth of larvae.
3. Dense shade may make the breeding place unfavourable because of the destruction of grass along the margins.
4. Dense shade may alter the composition of the fauna and flora.

5. The dense growth of plants covering breeding places may act as a mechanical barrier.

Muirhead Thomson tried to understand the role of each of these causes.

By carefully designed studies using a sensitive light measuring apparatus he was able to compare the light intensities in the open and under certain types of shade during different phases of the moon. He found that *Duranta*, a small leaved shrub, was a more effective shade-producer during the day than *Tītaput*, a large leaved shrub. But at night-time variations of light and shade were indistinguishable except on bright moonlit nights.

To test the effect of shade alone on breeding, a uniform stretch of drain was selected in which *A. minimus* larvae were breeding continuously. It was divided into two stretches each 50 feet in length; the first part was left untouched, and the second part was shaded by means of simple roof of bamboo matting fixed about four feet above the drain, both the ends being quite open. The shade produced was not dense enough to interfere with the growth of vegetation along the edges of the drain. Over a period of one month it was found that 179 *A. minimus* larvae were collected in the shaded part and only 67 larvae in the unshaded parts. This preliminary experiment seemed to indicate that shade was actually attractive to *A. minimus* larvae.

In a second experiment, the same shaded part of the drain was converted into a very dense shade by super-imposed thatch hanging down to the ground along both sides. Mosquitoes, however, had access from the two ends. The shade was so dense that the vegetation underneath soon began to die. But in order to keep this factor constant the grassy edge was kept renewed over a period of four weeks. 894 *A. minimus* larvae were found in the shaded part while only 276 were found in the unshaded part. The experiment confirmed that with the grassy edge intact the part with heavy shade was much more favoured than the unshaded control part. By comparing the light intensities in the two parts, it was apparent that light by itself could not have played any part in the control of breeding places by dense shading.

In another experiment in which vegetation in the heavily shaded part was allowed to deteriorate to make the edges practically bare, 1,445 larvae were collected in the shaded section and 1,601 in the unshaded section showing that in the absence of vegetation the shaded part was not more attractive than the unshaded part.

(c) The influence of water movement on the selection of breeding places: In a further experiment, the number of *A. minimus* larvae taken from an untouched control with some vegetation was ten times greater than that along the bare edge under shade. Further, the majority of the larvae under the shade were of young stages whereas in the control stretch of the stream larvae of all stages were taken. When in the garden drain vegetation was cleared away from a 50-foot stretch, only one larva was found in the cleared section as against 152 found in the uncleared control. It was therefore seen that clearing of vegetation and exposing the water to sunlight resulted in the end of breeding. The thick grassy edge of a typical *A. minimus* breeding place not only provided shade attracting the female but also

acted as a powerful hindrance to the flow of water and produced a zone of still water along the edge.

In laboratory experiments where gravid females were given a choice of depositing eggs in a shaded and unshaded portion of a cage at night, three times more eggs were laid in the dark cage than in the lighted cage. Muirhead Thomson concluded that shading of a breeding place renders it temporarily very attractive to the gravid female but the subsequent disappearance of vegetation puts an end to breeding.

(d) Flow of water: A large number of observations made by Muirhead Thomson showed that although *A. minimus* breeding was associated with running water, the larvae actually lived in still water provided by the grassy obstruction. It was also observed that there was no inhibition of egg laying in a still-water pocket along the bare edge of a drain. He carried out several field and laboratory experiments and showed that ovipositing females actually preferred to lay eggs in still water and avoided flowing water, even when the velocity was as low as 0.05 foot per second. In an experiment in which gravid females were given a choice of still water and water flowing at 0.05 foot per second, 67 per cent of the eggs were laid in still water. Surface ripples did not seem to influence the egg laying females.

When the water movement exceeded two feet per second the larvae were flushed away whether they were first stage larvae or fully grown fourth stage larvae. It appeared that *A. minimus* was not exceptionally well adopted for life in running water being only slightly more resistant to flow than other species such as *A. aconitus, A. maculatus* and *A. nigerrimus.* It was, therefore, clear that the presence of larvae in the streams and drains was mainly because of the shelter provided by the grassy margins which create practically still water pockets along the edges.

(e) The influence of water temperature on the choice of breeding places: In a series of studies on the temperatures of water of breeding places of *A. minimus,* Muirhead Thomson has made some very significant observations. They can be summarized as follows:

Though there are wide fluctuations in day-time temperatures of waters of different types of breeding places, the differences at night are so slight that water temperature by itself could not be the deciding factor influencing the gravid females. In laboratory experiments when gravid females were given a choice of water temperatures between 23° C and 30° C, there was no marked preference. However, when given a choice of between 30° C and 35° C the cooler water was preferred. The gravid female avoided high water temperatures, i.e., higher than those normally found in breeding places at night.

The highest temperatures recorded in natural waters, viz., 43.7° C and 43.8° C, were in rice fields with shallow water. At 15.00 hours on a sunny cloudless day in July the temperatures of some breeding places were: streams 34.° C, borrowpits 40 to 42.5° C, grassy rice fields 38° C to 42° C. In extensive studies in the favourable breeding places of *A. minimus,* the maximum temperature never exceeded 35° C. In unfavourable places such as tanks, it reached 40.7° C in August. Borrowpits, small puddles, hoof marks, etc., very similar to rice fields, had temperatures of about 42° C.

In experimental conditions in the laboratory, Muirhead Thomson found that the thermal death point, for *A. minimus* was 41° C. Thermal death points for other species were: *A. insulaeflorum* 40° C, *A. hyrcanus* 43.5° C, *A. barbirostris* 43.5° C, *A. culicifacies* 44° C and *A. vagus* 45° C. These findings explain the observations made all over India that species like *A. culicifacies, A. vagus* and *A. nigerrimus* can breed prolifically even in small or shallow water collections exposed to sun, such as borrowpits, shallow puddles, hoof marks and fallow and freshly planted rice fields. Therefore, high temperature of water in breeding places is one of the major factors for the absence of *A. minimus* larvae in shallow still waters. But in the side pockets of stillwater adjoining (and connected to) a stream or drain temperature is much lower due to the constant replacement of water and hence they can sustain a good larval population of *A. minimus*.

The development of eggs and pupae was studied at different temperatures and it was found that at 35°C development was still possible for eggs but not for pupae.

(f) Chemical composition of water:

The chemical composition of water and the influence of organic pollution and silt on the breeding of *A. minimus* were also studied in detail by Muirhead Thomson. He did not find any constant differences in the dissolved oxygen content between river water and tank water either during day or night. Free and saline ammonia was seldom present except as traces. Albuminoid ammonia and the tidy figure (oxygen absorbed from permanganate) were found to be the most valuable indications of the total organic matter and of the degrees of pollution. These were lowest in the type of water selected by *A. minimus*. Gravid females could not distinguish between water from the normal breeding places and water in which they did not ordinarily breed, and the differences in composition did not affect the growth or mortality of larvae. *A. minimus* adults were very sensitive to pollution by cut vegetation and they were able to distinguish the presence of rotting vegetation but the larvae could develop in such waters, though these were avoided by the gravid female. Therefore, the absence of breeding in places with cut vegetation or herbage packing was most likely the result of the selection made by the gravid female. Silt water neither prevented the eggs being laid nor the successful growth of the larvae. Therefore silt by itself, contrary to ideas previously postulated had no lethal effect on *A. minimus* larvae. The absence of *A. minimus* larvae from waters with heavy silt could be due to other causes.

Extending his investigations on the relationship of breeding of *A. minimus* to the composition of water and influence of organic pollution Muirhead Thomson concluded that the female mosquito is sensitive to organic matter equivalent two or three parts per million of oxypermanganate absorbed from waters.

In summary of the entire work it appears that the prevalence of *A. minimus* in certain types of breeding places and their absence in other could be due to several factors both connected with the reactions of the gravid females and also the conditions in the water favourable for the larvae. Muirhead Thomson concluded "the problem must rest here, until further experimental work shown whether the limiting factor in this case is really the low oxygen content, or whether it is a difference in

the quality of the organic matter which determines the behaviour of the mosquito."

The extensive studies by Muirhead Thomson have shown clearly how essential it is to analyse the various factors which can directly or indirectly influence the prevalence of any species in different types of breeding places.

A question often posed is: How do adults of *A. minimus* persist through cold weather. Muirhead Thomson (*loc. cit.*) found that eggs, larvae and pupae continued to develop even at the lowest water temperatures likely to exist in nature, which was about 15° C in January. During that month there were indications of substantial output of females, though the larval density was only about 1/10 of what was found in April and the output was 30-fold less. Perennial rivers, seepages and small streams are the breeding places during the cold weather. The work of Rice and Mohan (1936), referred to earlier is pertinent in this connection.

Relation to Disease

Several extensive series of dissections have been made and *A. minimus* has been found to be a vector all over its range of its distribution of the Oriental region. Some of the important results in India are:

Locality	Author		Sporozoite rate per cent
Assam	Anderson & Viswanathan (1941)		2.8
Assam	Viswanathan *et al.* (1941)	..	1.4
Bengal	Iyengar (1939)	..	2.5
Bengal	Iyengar (1940)	..	9.3
Bengal (Darjeeling)	Gilroy (1939)	..	1.4
Jeypore Hills	Senior White *et al.* (1945)	..	4.3
Singhbhum:			
Houses	Senior White & Das (1938)	..	3.9
Human bait	Senior White & Narayana (1940)	..	5.6
Outdoor shelters	Senior White *et al.* (1945)	..	15.4

Many other similar series of dissections have been made in Assam, Bengal and East Central India with more or less similar results. The season of transmission is throughout the year except in the coldest months. In the areas where streams and channels are flushed away during heavy rains the season commences mainly after the monsoon. Before the importance of *A. balabacensis* in malaria transmission had been recognised in Assam, *A. minimus* was considered to be the primary vector, but it is now necessary to determine more precisely the disease transmission role of *A. minimus*.

In the Chittagong hill tracts of Bangladesh, Khan and Talibi (1972) recorded *A. minimus* as a probable vector with two peaks of transmission. In the Terai area of Nainital District, Uttar Pradesh, where *A. minimus* was supposed to be the chief vector, Srivastava and Chakrabarti (1952) after making an epidemiological survey

found that *A. minimus* was neither the chief vector nor the sole vector in that area. They based their conclusions on several reason:

(a) Low prevalence of larvae and insignificant adult densities;
(b) Little rise during the seasons of high transmission;
(c) No infections found in either guts or glands; on the other hand they found *A. fluviatilis* to be the confirmed vector between 1949 to 1951.

Sen (1948) made a review of the previous dissection records in Bengal and showed that there were three vectors in Bengal, *A. sundaicus* in the coastal region, *A. philippinensis* in the deltaic area and *A. minimus* in the foothills. He found one out of 17 *A. minimus* dissected positive. In Tripura State and in NEFA *A. minimus* has been incriminated as a vector (Misra & Dhar, 1955 and Misra, 1956). *A. minimus* has also been incriminated as a vector in several neighbouring and Southeast Asian countries.

In Nepal, Brydon, *et al.* (1961) had confirmed that *A. minimus* was the primary vector in the hyperendemic forest belt of Terai and inner Terai but not in the upper hilly regions. In 1966, Shreshta also stated that *A. minimus* was the primary vector in the hyperendemic belt. But in more recent studies (Pradhan *et al.*, 1970) *A. minimus* does not find any mention. On the other hand, *A. fluviatilis, A. maculatus willmorei* and *A. annularis* are now regarded as the vectors.

With the recent dwindling of *A. minimus* populations in India, the vectorial status of the species needs reconsideration.

In Burma, *A. minimus* is regarded as the most important vector responsible for the hyperendemic conditions. The total infection rates have varied from 1.3 per cent to 3.7 per cent. It is essentially a mosquito of the hilly regions and has a wide distribution all over the country. Khin-Maung-Kyi (1971) has not referred to any change in its prevalence or habits though it is still quite susceptible to DDT, except in some changes in the biting time.

In Thailand, it continues to be a very important vector along with *A. balabacensis* but the extensive use of DDT seems to have changed its habits; there is more outdoor resting and outdoor feeding than had been previously known.

Harinasuta *et al.* (1976) have pointed out that *A. minimus* is a major vector of malaria in the following Southeast Asian countries: Burma, Kampuchea, Laos, Vietnam and Thailand. But they make a comment that it "tends to disappear with the use of DDT in Thailand". The work of Ismail *et al.* (1978), however, indicates that it is still an important vector though it has changed its habit from endophily to exophily.

In North Vietnam *A. minimus* is still regarded as the chief vector of malaria. The distribution of *A. minimus* seems to be in the high mountain regions where it accounted for 17.1 per cent of all the anopheline species collected. The other common species were *A. vagus, A. sinensis, A. kochi, A. aconitus* (Leysenko, Vengy, Vankhyu and Tkao, 1961). In another study Spring *et al.* (1961) made over 4,000 dissections of *A. minimus* and found 1.14 per cent sporozoite rate, *A. vagus* playing a secondary role with a sporozoite rate of 0.6 per cent.

The variety, *flavirostris*, is well known as the main vector in the Philippines. It

has always been characterized by its outdoor resting habits.

There is no evidence to suggest that *A. minimus* takes part in the transmission of non-human malaria or in filariasis.

Control

Early efforts of control of *A. minimus* by anti-larval measures particularly shading and channeling have already been described. These methods had been employed for many years in the tea plantations of Assam. Further, introduction of silt laden water into streams had also been recommended. The exact mechanism of action had remained uncertain until the classical work of Muirhead Thomson on the behaviour of the adults and larvae in relation to the breeding places. Shading resulted in the disappearance of vegetation in the channels and led to an increased velocity of the flow of water. The larvae could not survive in open flowing water without vegetation.

With the introduction of pyrethrum space spraying, Viswanathan (1942) carried out a weekly spraying of pyrethrum extracts in the tea estates of Assam. He found a reduction of the sporozoite rates. Later Viswanathan (1942) reported that *A. minimus* had practically disappeared.

However, with the availability of DDT extensive work in the control of *A. minimus* has been done. Kar (1950) confirmed that in Assam where *A. minimus* was regarded as the main vector, DDT indoor residual spraying was very effective. The work was done in 1946. The subsequent extensive work by the National Malaria Eradication Programme has had a very good effect on *A. minimus* and till now there is no evidence of resistance to DDT in this species to the extent of interfering with the programme. Puri and Krishnaswami reported the successful use of DDT against *A. minimus* in the tea gardens of the Darjeeling Terai (Puri and Krishnaswami, 1947 and Krishnaswami, 1952). In other countries, too, such as Burma, Thailand, and Malaysia, there have been excellent control with DDT. However, malaria persists in many parts of India where *A. minimus* was formerly regarded as the chief vector. This is certainly due to the importance of other species such as *A. balabacensis* and *A. fluviatilis* which had not been previously recognized.

Addendum

B.A. Harrison (1980) has made a comprehensive revision of the Myzomyia series from the Oriental region. This monograph would have to be studied in detail but from brief overviews of the findings kindly provided by Dr. Harrison the following summary can be made:

"The type-specimens or type-series for 17 nominal taxa were located and examined. The location of several types is corrected. The pupae of *pampanai* and *varuna* and described and illustrated for the first time. Morphologically deformed variants of *aconitus* and *minimus* adults are described. *A. culicifacies adenensis* and *jeyporiensis* var. *candidiensis* are synonymized. The junior primary homonym *listonii* Liston, is necessarily considered a rejected name. *Pyretophorus jeyporensis* Theo-

bald is considered a junior secondary homonym of *Anopheles jeyporiensis* James. The authorship of the species previously cited as *brahmachari* Christophers by most writers is corrected to McKendrick and Christophers. The name *aconita* var. *merak (cohesia)* is considered an available name and shown to be a synonym of *flavirostris* instead of *minimus*. Lectotypes are designated for *adenensis, albirostris, christophersi, culicifacies, formosaensis, jeyporensis* and *listoni."*

"The oriental Myzomyia series is probably the most economically significant assemblage of anopheline vectors of human malaria and filarial parasites in the Indian and Southeast Asian subregions of the Orient. Besides, *culicifacies, jeyporiensis* and *majidi*, the series also includes the 8 species in Minimus Species Group, *i.e., aconitus, filipinae, flavirostris, fluviatilis, mangyanus, minimus, pampanai* and *varuna*. A total of 9 of the 11 species have been confirmed as vectors of human malaria parasites in the Orient (includes *aconitus* and *minimus* in Thailand), and 7 of the species have been confirmed as vectors of human filarial parasites in the orient."

Aconitus and *minimus* were found to be extremely variable species, with considerable character overlap.

"Anopheles aconitus and *minimus* were colonized for the first time and 122 cross mating experiments were carried out. These studies revealed that *aconitus* and *minimus* have considerable genetic incompatibility, *i.e.*, nearly all F_1 hybrid specimens were inviable. These results essentially rule out hybrid specimens as being responsible for the numerous observed variants."

"Considerable emphasis was placed on the bionomics of these species. The biology and habitat requirements of the adults and immatures were reviewed and summarized. The known parasites and pathogens for each species were also reviewed. Additional data were also presented that show *minimus* is much more attracted to feed on bovines in central Thailand than previously suspected. In paired comparative studies, *minimus* was more frequently collected biting bovines than man. These results are very similar to those obtained for *aconitus*, a known zoophilic species, in the same study village. The significance of these findings is that although both species are vectors of malaria parasites in Thailand and very common in the study villages, the prevalence of malaria in the villages was very low."

"The medical significance of the oriental Myzomyia species was reviewed with emphasis placed on the 6 species in Thailand, particularly the confirmed vectors, *aconitus*, and *minimus*. *A. culicifacies*, a primary vector of malaria parasites in the Indian subregion, was found to be more common in Thailand than previously suspected. Since this species is known to be resistant to DDT in Thailand, it is considered a potential vector. The other 3 species, *jeyporiensis, pampanai* and *varuna*, are not considered potential vector threats in Thailand because of their limited distributions and low population densities."

Anopheles varuna Iyengar, 1924

Type locality: Near Calcutta, India.

Type: Location unknown. Director, Zoological Survey of India, now states that

the type is not in its collection.

Taxonomy: The species was being identified as *A. minimus* till Iyengar (1924) described it as a distinct new species. The older records in Southern India of *A. minimus* are now in doubt.

Distinguishing characters: Adult *A. varuna* is a medium sized mosquito of a fairly dark appearance. The adult can be distinguished from its close relatives in India, *viz., A. minimus, A. fluviatilis* and *A. aconitus* by the combination of the following characters:

Wing: Inner quarter of costa without any pale interruption and without even a few pale scale;fringe spot on vein-6 absent; palpi with two broad apical bands separated by a narrow dark area; usually proboscis flavescent in the apical half, sometimes faint. For other characters see the Key. In larvae the hair-0 on the abdominal segments arises from the tergal plate.

Distribution: India, Nepal, Sri Lanka, Bangladesh, Burma, Thailand and South China (See Venkat Rao, 1961). Occurrence in Thailand recently confirmed (Harrison, 1980).

In India: Prevalent in all eastern, south-eastern and southern zones. Scattered prevalence in Gujarat. Not found in the Andamans but recorded in Lakshadweep.

Prevalence

A. varuna occurs both in the foothills and plains. It has been found at high altitudes, as for instance in Nepal where it is, however, very scarce. It is also found at sea levels as for instance in Rameswaram Island in Tamil Nadu (Sitaraman *et al.*, 1978). Much of the information on the habits and habitats of *A. varuna* has come from the work of Senior White, Venkat Rao and colleagues in east-central India.

A. varuna forms a substantial part of the adult anopheles populations in the Satpura ranges of Madhya Pradesh (Senior White, 1941). Actually it was more prevalent than *A. fluviatilis* and *A. minimus.* In Visakhapatnam, *A. varuna* formed 75 per cent of the total anopheline population in villages. In other areas in India where the species occurs it forms a much smaller proportion in the total population.

Adult Bionomics

Adults have long been known to be readily collected in houses and cattlesheds. There is also evidence that a considerable degree of outdoor resting occurs. Venkat Rao (1961) analysing the resting habits in different areas of east-central India, has stated as follows:

(a) *Jeypore hills:*	Mostly in houses during the earlier part of each gonotrophic cycle and later in outdoor shelters. Very few specimens in cowsheds.
(b) *Singhbhum hills:*	Many specimens in houses and cowsheds but many in

outdoor shelters.

(c) *Eastern Satpuras:* No evidence of resting in outdoor shelters.

(d) *Visakhapatnam area:* Overwhelming majority of specimens found in a special type of cowshed away from human habitations.

(e) *Bengal and Orissa:* Evidence not conclusive.

In Thanjavur District, Tamil Nadu, Russell and Ramachandra Rao (1941) collected adults in houses, mixed dwellings and animal dwellings in about the same densities but no outdoor collections were attempted. Senior White and colleagues (1937-38) observed that in their area human habitations were preferred to cowsheds but they were dealing collectively with three species, *viz., fluviatilis, varuna* and *minimus,* which had similar breeding, feeding and resting habits. Senior White and Venkat Rao (1943) made observations in a special type of hut in which the edge of the roof was brought down practically to the ground level so that only one exit, *viz.,* front door, was available. Human and buffalo baits were used. Collections to depletion were made daily for many months. They collected over 11,000 *A. varuna* specimens of which 762 were from the human baited hut and 10,567 from the buffalo baited trap. While this study did show that both man and buffalo were attractive, it cannot be stated what the relative degree of attraction was. In the present author's experience, a full sized buffalo or cow offers much more surface for feeding than a man and it would be difficult to make direct comparison. Senior White *et al.* (1945) again showed that in Singhbhum hills 2,118 *A. varuna* adults were collected from outdoor shelters (steep banks of small streams close to villages) and only 90 from unsprayed houses and 66 from sprayed houses. On the other hand, numbers of *A. fluviatilis* collected at the same time were 3,649 in unsprayed houses, 151 in sprayed houses and only 91 in outdoor shelters, suggesting marked differences in the adult behaviour of the two species. Some indication of a low degree of outdoor resting may be obtained by the studies made by Russell and Ramachandra Rao (1941) in the magoon type traps. They found 773 adults in houses and cattle sheds as against only 66 were collected in the magoon trap quite unlike *A. nigerrimus* of which 123 were found in houses and cattlesheds and 5,339 in the traps, indicating that *A. varuna* in the district preferred to remain in indoor shelters after feeding while *A. nigerrimus* left the shelters after feeding.

Biting habits: Most of the information regarding the biting habits of *A. varuna* was provided by the studies of Senior White and colleagues in east central India. The time of entry into houses for feeding was considered to be similar to that of the other members of the *"minimus"* group in that region, i.e., the numbers entering between midnight and dawn being much higher than before midnight. One cannot be certain of this because, as shown above, there was a very marked degree of difference between the habits of *varuna* and *fluviatilis* in the Visakhapatnam area. In Burma, (Khin-Maung-Kyi, 1971) the biting time on cattle seemed to be between 18.00 and 22.00 hours.

Feeding habits: Though generally regarded as anthropophilic there have been some variations in behaviour. According to Venkat Rao (1961) *A. varuna* resembles *A. fluviatilis* and *A. minimus* in its marked preference for human blood whether the specimens were collected in houses or in cattle sheds, but in Singhbhum hills and Visakhapatnam those collected from houses had much higher anthropophilic indices than those collected in cattlesheds as the following figures would show:

Species	Locality:	Houses			Cowsheds			Out of doors		
		No.	M.	AI	No.	M.	AI	No.	M.	AI
A. varuna	Jeypore Hills	96	78	81.2	22	14	63.1	7	6	85.7
do	Singhbhum Hills	124	66	53.2	114	7	6.1	347	14	4.0
do	Visakhpatnam area	95	55	58.5	779	91	9.1	—	—	—

No. = Number examined M = Number with human blood. A.I. = Anthropophilic indices.

In Visakhapatnam area and Singhbhum hills *A. varuna* seemed to be more zoophilic. Whether this indicated existence of two biological races cannot be stated as zoophilic indices found may be also due to differences in the prevalence and availability of cattle for feeding.

In a recent survey in Bastar District of Madhya Pradesh by Hussainy (1978) only 71 adults were collected out of 21,716 anophelines of all species. Analysing this small collection, Hussainy found that:

(1) the specimens were found mainly in forest areas;
(2) the feeding activities in general were completed before midnight;
(3) the species was more prevalent during the cold season;
(4) the altitude of occurrence ranged from 48 to 761 metres;
(5) 68 of the 71 specimens were collected at night and two were collected in cattlesheds during daytime.

Longevity: Direct information on the longevity of *A. varuna* either in the laboratory or in the field is not available, but inferring from the fact that the species has a high infection rate, it is presumed that it has a long life comparable to those of *A. fluviatilis* and *A. minimus*.

Gonotrophic cycle: While normally in the warmer months of July and August, *A. fluviatilis* feeds once in 48 hours (Senior White *et al.*, 1945), it was detected that there was a gonotrophic discordance i.e., ovarian development and the digestion of blood not proceeding at the same speed. Venkat Rao (1947) showed by dissection of the ovaries that *A. varuna* also may need repeated feedings for maturation of eggs. Similar phenomena of gonotrophic discordance or dissociation have been reported in other species such as *A. annularis*. No information is available on the length of the gonotrophic cycle in the cooler months. One can presume that it will be similar to that of *A. fluviatilis* and *A. minimus*.

Dispersal: As the breeding of this species has been found up to a distance of about half a mile from villages in east central India (Hazaribagh and Jeypore hills), it is presumed it to be the flight range of this species (Venkat Rao and Philip, 1947). There is no other experimental evidence.

Swarming and mating: No information.
Oviposition: No information.
Colonization: No colony of *A. varuna* has yet been reported to be established.

Larval Ecology

A. varuna is found to breed in a variety of breeding places, both in stagnant and flowing water. While Iyengar (1942) stated that stagnant fresh water in ponds and ditches provided the breeding places around Calcutta, according to Puri (1931), small streams and wells in North Kanara were the breeding places. Roy (1938) brought out differences from the observations of Puri. He said that the species was found in stagnant fresh water, tanks, ponds, etc. with algal and other aquatic vegetation. The most important feature, however, was that the larvae liked shade from overhanging branches of trees, etc. These findings are also very different from the observations of Russell and Ramachandra Rao (1940b) who found a high degree of breeding in wells in Thanjavur District. They found that there was no seasonal preference in the breeding, but the intensity of breeding was high during June when the water table was lowest in the month prior to the onset of the irrigation season. Perhaps this was because of the fact that deeper wells provided more shade than did open wells.

In Thanjavur District of Tamil Nadu, preference for wells was indicated by the fact that out of the 1,240 wells searched *A. varuna* was found in 623 (approximately in half the number). In a total of 6,033 searches of all types of breeding places, *A. varuna* was found 762 times out of which wells were 623 in number. Similarly out of 17,682 larvae of this species collected in all breeding places, 16,792 were in wells. A few larvae were found in other breeding places, such as irrigation canals and distribution channels and rarely in tanks, ditches, pools or rice fields. Therefore, *A. varuna* had by and large a predilection to breed in wells. In fact, in wells it was more prevalent than *A. culicifacies* and *A. subpictus*. There was no special preference between irrigation wells in the fields and the lined domestic type of wells in the villages.

In Visakhapatnam in Andhra Pradesh, *A. varuna* has been found to be breeding profusely in nallahs, irrigation channels and wells particularly kuchcha wells. In a survey made by Senior White and Venkat Rao (1943), *A. varuna* formed 50 per cent of all the species collected.

In Rameswaram Island in the southern part of India (Sitaraman *et al.*, 1978), *A. varuna* was found in association with *A. culicifacies* in the breeding places, particularly wells. It also occurred in open pits in sandy terrain in coconut and casuarina plantations and in brackish water. Though there is no special predilection for aquatic vegetation, *A. varuna* was among the 12 species of anophelines found in Bengal by Sen (1948) being associated with growth of many types of plants.

In Sri Lanka, Rajendram and Jayawickreme (1951a) found *A. varuna* and *A. vagus* breeding intensely in sand and rock pools. *A. varuna* was the predominant anopheline larva within the epidemic zone breeding places. In the year 1941, 1942,

1944, 1948 and 1949 *A. varuna* and *A. vagus* larvae outnumbered even *A. culicifacies* larvae. The adult densities of *A. varuna* were however slightly lower than those of *A. culicifacies*. In Burma, like *A. minimus* it breeds in slow moving streams with grassy margins, seepages and rice fields under strong sunlight and in clear water (Khin-Maung-Kyi, 1971).

Therefore, it seems that *A. varuna* may have several types of breeding habits in different areas. Whether these are the result of environmental factors or behavioural variations within the species itself cannot be judged.

Relation to Disease

All the positive dissections for plasmodium infections have been made in India. Commencing from Iyengar's work in 1939 in Bengal, dissections of *A. varuna* have been made in a number of places. Dissections have been made in Bengal, Assam, East Central India, including Satpura, Hazaribagh, Singhbhum and Jeypore hills in Orissa, Tamilnadu, Kerala and S. Kanara and North Kanara. A few dissections have also been made in other localities. It seems that infected adults have been found only in the coastal Andhra Pradesh (two gland infections out of over 11,000 dissected); in east central India (56 gut and 24 gland infections out of nearly 3,500 dissected); in West Bengal (one gut and four gland infections out of over 1,000 dissected); and in Kerala (10 gut infections and seven gland infections out of a total of 429 dissected). Dissections in Bengal were made by Iyengar, Sen and Roy, in east central India by Senior White and colleagues, in Andhra Pradesh coast by Senior White and colleagues and in Kerala by Mathew (1939). In Kerala, Covell and Harbhagwan (1939) found no infections in *A. varuna*. No infections were also found by Jaswant Singh and Jacob (1944) and Viswanathan (1950) in North Kanara, by Russell and Ramachandra Rao (1943) in Thanjavur and by Viswanathan (1941) in Assam. Therefore, the positive findings in Kerala need confirmation. Even in coastal Orissa, Covell and Pritam Singh did not find any infections.

Therefore, it is only in the hilly and forested east-central India that the species seems to have any significant role as a malaria vector and perhaps to a certain extent in deltaic Bengal and in Visakhapatnam coast of Andhra Pradesh. In east-central India positive dissections have been reported as shown in Table 24 (adapted from Venkat Rao, 1961).

Table 24. *A. varuna* dissections, East Central India

Locality	Year	Number dissected	Gut positive	Gland positive	Sporozoite rate
Jeypore Hills	1935-37	506	34	17	3.3%
Singhbhum Hills	1938-45	2,530	19	5	0.2%
Hazaribagh Hill tract	1941-42	131	3	2	1.5%
East Satpura Hills	1948-49	326	—	—	3.7%

In the same areas *A. fluviatilis* and *A. minimus* were also found infected. Obviously in the above areas *A. varuna* was a vector of significance either equally or to a slightly less extent than *A. fluviatilis* or *A. minimus*. In a recent study in Rameswaram Island, during a severe malaria epidemic no adults were found in houses though there was intense breeding. However, out of 67 *A. varuna* dissected from those collected biting on cattle outdoors, no positive was found, but one infection was found in *A. culicifacies*.

A. varuna has to remain a suspect wherever it occurs in large numbers. It is being suspected in Lakshadweep.

There is no information regarding the role of *A. varuna* as a vector of animal malaria or of human or animal filariasis.

Anopheles culicifacies Giles, 1901

Type locality: Hoshangabad, Madhya Pradesh, India.

Type: British Museum of Natural History, London. The male type is stated to be really a specimen of *A. turkhudi*. Harrison (1980) states that he has deposited lectotypes for several Myzomyia as including that of *A. culicifacies*.

Taxonomy: There are three synonyms, all reported from India, *viz., indica* Theobald, 1901, of Madras; *Listoni* Giles, 1901 from Ellichpur, Maharashtra; and *punjabensis* James, 1911 from Punjab. There is, however, a recognized subspecies *adenensis* Christophers, 1924 occurring in Aden, Yemen Trucical Oman, Socotra, Ethiopia, Eritria, etc., but not in India.

Distinguishing characters: Adult *A. culicifacies* is a small to medium sized mosquito. It has a culex-like posture when resting and hence the name. Such a culex like posture is shared by it with some members of the subgenus *Anopheles* such as *A. aitkenii, A. insulaeflorum, A. culiciformis* and *A. sintoni*. From its close relatives in the series *Myzomyia, A. culicifacies* adults can be distinguished by a combination of characters; a pale interruption in the basal quarter of the costa with an opposing dark spot on vein-1; vein-3 predominantly dark; only two fringe spots on veins 4-2 and 5-1 and no fringe spot on vein-6; mesonotum uniformly light coloured; leaflets of phallosome more than 3 on each side.

Larvae: See main key.

Distribution: Very wide distribution from Afghanistan, Iran, Trucical Oman, Bahrein, Pakistan, through India and Sri Lanka, Nepal, Bangladesh, Burma, Thailand, 'Indo-china' and southern China. (No records in Indonesia, Malaysia, Philippines or Taiwan).

In India: It occurs in all mainland zones including Kashmir and high elevations in the Himalayas. It does not occur in the Andamans or Lakshadweep.

Prevalence

A. culicifacies is a common mosquito in most parts of India. It is a malaria vector of great importance and has been found infected throughout India and some of the countries both to the west and east of it. It is abundant in the plains but is

less prevalent in the eastern parts of the country. Its prevalence is rather low in the hilly areas particularly in the Western Ghats. Bhatia and Krishnan (1961) thought that the original home of the species lies in India between Delhi and Pattukottai.

Formerly it was not considered a malaria vector of any significance in the part of India east of a line drawn from Lucknow to Visakhapatnam. Though this is not strictly correct because infected specimens have been found in the east also and even in Burma, the statement is generally applicable. It is either a weak vector or not a vector at all in most areas of the eastern region.

Greatest prevalence of *A. culicifacies* is in the areas in the plains receiving fair amount of a rainfall or irrigation. Even in desert or semi-desert areas of Rajasthan, northern Gujarat, Kutch, etc. as well as in the Punjab high numbers of *A. culicifacies* prevail in the monsoon and post monsoon months. Starting from negligible numbers the species multiplies to enormous numbers within the course of four to six weeks causing intense local or widespread regional epidemics of malaria, described by Covell and Baily (1930).

A. culicifacies is also known to occur at high altitudes and even to transmit malaria in such places, though not to the same extent as in the plains. There are many early records of occurrence at high elevations such as at Quetta (1,600-2,000 metres), Nainital (2,130 metres), Kashmir (3,000 metres) and Murree Hills, (Pakistan 2,500 metres). In recent surveys, Jacob (1950) found the species at an altitude of 2,000 metres in Kashmir, Srivastava (1964) in Almora (3,130 metres) and Wattal *et al.* (1973) in Nainital District (1,600 metres). Ramachandra Rao *et al.* (1973) found the species all along the Himalayan region at elevations of upto 1,130 metres. Iyengar (1954) collected the species in Kabul region of Afghanistan (1,500 to 2,000 metres). In southern India, the species has been found at elevations upto 1,000 metres in the Nilgiris (Russell and Jacob, 1942).

Adult Bionomics

An excellent review of literature on the biology of *A. culicifacies* till 1940 was by Afridi and Puri (1940) followed by a second article on original observations of Afridi, Majid and Imdad Ali Shah (1940). The first article provides the basic information to serve as the starting point for the description of the behaviour and ecology of the species. The article by Bhatia and Krishnan (1961) in the *Malaria Vectors in India* is full of valuable information.

Pre-1940 studies: The information provided by Afridi and Puri regarding the adult bionomics is summarized below:

(a) Seasonal prevalence: They divided India (pre-partition) into 7 divisions and presented information on the seasonal prevalence. They were:

Division	*Seasonal prevalence*
1. North West Frontier Province and Baluchistan	. . Peak prevalence in September and October.
2. West and central Punjab	. . Two peaks of prevalence, one during

		March/April and the second during September-October.
3.	Southeast Punjab, Sind, western United Provinces, Kutch, north Gujarat, South Bihar, Orissa, Jeypore Hill tracts, Hyderabad State and part of Central Provinces.	Throughout the year, but higher densities in March/April and September/October.
4.	Eastern United Provinces, riverine tracts of UP and north Bihar.	—do—
5.	Eastern Terai (UP), Assam, Bengal, western Coorg, Malabar, hill tracts of south west Coorg.	Peak in December/January.
6.	Madras, Mysore, northern Madras, low-lying parts of Malabar.	Peak in December/January.
7.	Vizagapatnam, and parts of north Madras and Central Provinces.	Throughout the year, with high prevalence from July to December.

The geographical and political names of divisions were those current prior to partition of India. Nothing was mentioned of the old Bombay State areas now in Maharashtra.

(b) Resting habits: *A. culicifacies* was commonly regarded as a domestic species. As a general rule adults preferred to rest in cattle sheds and houses during daytime but also sheltered in straw, mudcakes, etc. near stables, an indication of outdoor resting. The adults were also taken from dense vegetation (Gill, 1917) and under bushes (Perry, 1914). Cattle sheds generally yielded a larger number of resting adults than human dwellings (Senior White, 1937, Senior White and Das, 1938, Perry, 1914), but some observers like Timbers (1935) found the reverse to be common in Birbhum, Bengal.

(c) Nocturnal habits: In Birbhum, Bengal, Timbers (1935) found the catch from 21.00 to 23.00 hours to be 2.6 times more than the catch between 03.00 and 05.00 hours. Nursing *et al.* (1934) in Mysore State, and Senior White (1937) in Jeypore hills, however, found two thirds of the night catch between 04.00 and 06.00 hours. Afridi and Puri (1940) in Delhi found the number from 22.00 hours to midnight far exceeding the collections in the earlier part of the night. Afridi and Puri emphasised that as the methods adopted by different workers were not uniform, it was difficult to postulate the existence of biological races on these results only.

(d) Feeding habits: Studies prior to 1936 were mainly based on impression and many conflicting reports were published. Till the technique of precipitin test of the blood in the stomach of mosquitoes was developed, observations were mainly based on resting preferences. It was, however, known in many localities where *A. culicifacies* was a vector that it was not a habitual feeder on man. For example in Poona (new Pune) Barber and Rice (1938) showed that among the stomach bloods examined the human blood positives were only 3.4 per cent. Afridi *et al.* (1939) found in

Delhi a rate of 1.8 per cent. Senior White (1938) reported no human blood in collections made from cattle sheds in Jeypore hill tracts, while it was 7 per cent in the collections made in houses.

Man biting propensities also varied according to season and also to the ratio of numbers of humans and cattle in a given locality. The latter is obviously the most important factor as shown by the study of Russell and Jacob (1939) who found an anthropophilic rate of 80 per cent in the Ennore-Nellore area, north of Madras City, where cattle were scarce. Afridi *et al.* (1939) also made similar observations in three villages near Delhi

Seasonal variations in man-biting were also known. In Delhi a higher rate of man-biting was noticed during the season when temperature and humidity were most favourable for the production of the species. There was some evidence in Peshawar, now in Pakistan, (Richmond and Mendis, 1930) that a high rate of man-biting occurred when the humidity was higher, but Barber and Rice (1938) in Pune found that the biting activity was less in the monsoon months than in the dry months. They thought that the sudden lowering of temperature due to the onset of the monsoon resulted in a slowing in all biological activities.

Further Studies (Post 1940): After the above brief summary of the review by Afridi and Puri (1940), the adult bionomics will now be dealt with in detail, particularly of the work done subsequently. Some elaboration of the earlier observations will be necessary but generally work done prior to 1940 will not be dealt with at length.

Seasonal prevalence: As already noted by Afridi and Puri the actual seasons of prevalence varied from place to place. In northern India with the onset of the monsoon there were numerical increases in *A. culicifacies* in Budhakhan, a village near Karnal (Afridi *et al.*, 1940). Mosquitoes belonging to the earlier generations dispersed freely upto 500 yards (450 metres). By September the numbers had gone down and there was a slowing of biological activities. The ovarian cycle increased from 3 to 4 days to 7 to 8 days. However, the range of infiltration and dispersal continued to be about 450 metres. From the middle of November and to the end of December the biological activities were further slowed down and the ovarian cycle covered 4 to 8 days and the average length of life which used to be 5 to 6 days, during the earlier monsoon, increased to 12 to 15 days. The movement also became restricted. In January and February, with minimum air temperatures between 4.4° to 10.0° C, there was a sudden fall in the numbers of *A. culicifacies* and those captured during these months were fairly old specimens. Their movements were restricted to short flights between neighbouring houses. They were often engorged with fresh blood and their ovarian development did not altogether cease. There was no evidence of hibernation or gonotrophic dissociation. Pupae were still collected from canals. However, there was certainly a few over-wintering females. In March the first generation of the year emanated from the over-wintering females. Thereafter the number of *A. culicifacies* adults increased sharply, till further spurt took place after the onset of the monsoon in June and July (Afridi, Majid and Imdad Ali Shah, 1940).

According to Pal (1945) the highest prevalence in the Punjab was in June, August and September, that is the hot humid months.

In southern India where extensive studies on *A. culicifacies* were made in a newly irrigated area, Russell and Ramachandra Rao (1941a) have reported that in that area, where there is really no cold weather, *A. culicifacies* was prevalent during all parts of the year, but the highest prevalence was found from June to November coinciding with the season of irrigation. Very negligible densities of less than 0.5 per man hour occurred in the months of April and May but rose to 40 to 50 per man-hour during the irrigation season. They found no correlation between this rise and temperature, relative humidity, saturation deficiency or rainfall. The marked upward trend in June and July was entirely due to a great increase in the number of available breeding places. Later in the year, particularly October to November, there was a decline in the number of adults and larvae of *A. culicifacies*. All breeding places holding irrigation water continued to be full but for some reason both intensity of breeding and density of adults sharply declined. The relative humidities were higher during this season. The months of December and January, which is the last quarter of irrigation season, was characterized by comparatively low prevalence of *A. culicifacies*. These were also the coolest months but the temperature was not low enough to affect the ability of *A. culicifacies* to transmit malaria. Apparently there was a seasonal effect but other experiments have shown (Russell and Ramachandra Rao, 1942d) that the decline was more probably due to the aging of the breeding places and the growth of vegetation rather than to season. When fresh borrowpits were dug in December, the species readily laid eggs in them. From February onwards till the end of May, the irrigation water was completely absent and the few *A. culicifacies* which persisted were found in unusual breeding places such as tanks, (unsuitable), wells (suitable but not many) and river bed pools (highly suitable, but rare). The north east monsoon which occurs in this area between November and January had apparently no stimulative effect on the prevalence of *A. culicifacies* if at all it was adverse. According to the classification of Afridi and Puri (1940), Thanjavur District should fall into their category (f) with maximum prevalence of *A. culicifacies* in the cooler months and the season of low prevalence from June to October, but the entry of irrigation in this area commencing from 1935 had completely altered the picture. Very interestingly the pattern of prevalence of *A. culicifacies* in south east India proposed under type (f) by Afridi and Puri is substantiated by the prevalence of *A. culicifacies* in the Ennore area north of Madras City. Though meteorological conditions in the cooler months in Ennore were not very different from those in Thanjavur District, *A. culicifacies* had the highest prevalence in November and December when in Pattukkottai it was definitely declining.

Resting habits: While numerous observations all over India have confirmed the fact that large numbers of adults of the species rest in man-made structures during day, some outdoor resting has also been observed. For example Barber and Rice (1938) in Pune collected 52 individuals resting under culverts and other outdoor places out of a total 1,629 but the effort to make outdoor collections was rather limited.

In this connection, the experimental observations made by Shalaby (1970) in Panchamahals District of Gujarat are very interesting. In eight pit-shelters which he created, he found 3,377 adults of *A. culicifacies* during the course of one year, between October 1961 to September 1962. The females were in all stages of gonotrophic conditions indicating that it was not merely a casual habit of unfed or freshly hatched specimens; 55 per cent were unfed, 20 per cent freshly fed, 21 per cent half-gravid and four per cent fully-gravid. The present author made similar observations when he used earth-lined wooden boxes as artificial shelters in outdoor places in Thanjavur District. Many males and females were collected but as the numbers needed for other entomological studies were easily available in houses, outdoor resting was ignored.

The present author has also found on numerous occasions *A. culicifacies* adults resting in outdoor shelters. Not only was this found in certain localities in the forest, as in the Dhadgaon area of West Khandesh District in Maharashtra, where in the absence of any man-made structures, *A. culicifacies*, which was biting on forest workers engaged in charcoal manufacture, rested entirely out of doors, but also near Pune City where they were found resting in the crevices of rocks and under bushes.

The indoor resting habit of the species is very well known and generally many more adults are collected in cattle sheds and mixed dwellings than in human habitations. Practically every group of workers has made these observations. Two instances are however of interest. In the Pattukottai area, Russell and Ramachandra Rao (1942c) found that a higher proportion of adults even resting in houses had bovine blood in their stomachs indicating that though the feeding took place on cattle, mostly those tethered outside, they entered houses for daytime resting. In Afghanistan, Ramachandra Rao (1951) found that in the warm eastern region of Laghman, the vast majority of the people slept outdoors during night. Though most of the feeding took place in the open, *A. culicifacies* adults were found resting in large numbers inside houses during daytime. Therefore, there is enough evidence that a good proportion of *A. culicifacies* adults rest both outdoors and indoors during daytime but the exact proportions have not been determined.

In Sri Lanka, Ariaratnam (1955) found that cattle were usually tethered in the open as cattle sheds were seldom used and he observed that when cattle were tethered within a few yards of houses the catches in the houses were higher than on other days. He also concluded that mosquitoes might feed on cattle out of doors but subsequently enter the dwellings for resting. Reisen *et al.* (1976) in Lahore District of Pakistan observed that *A. culicifacies* rested mainly indoors and that both mated and virgin adults were found.

Inside the houses or cattle sheds, Pal (1945) observed that the unplastered walls of a room yielded large numbers of *A. culicifacies* than did rooms with the walls plastered. Many observations have also been made on the relative frequency of resting on surfaces such as those of clothes, umbrellas, furniture, firewood, etc. (Muirhead Thomson, 1951; Pal and Sharma, 1952; Ariaratnam, 1955, and others) but critical evaluation of the attractiveness of such surfaces have not been made.

Even regarding the preferential heights above ground selected for daytime resting there has been no general agreement.

In the experience of the present author while no part of the wall is free, there is a general preference for the roofs when they are at the normal height of 2.4 to 3.6 m above ground. As a matter of fact in a study in Maharashtra (unpublished) about 70 per cent of *A. culicifacies* were found resting on the underside of roofs of village houses while only 30 per cent rested on the vertical walls and surfaces of furniture, vessels, grain bins, etc. The excellent results of the programmes of DDT indoor residual spraying was attributed in Bombay State to the fact that, though the mud surfaces of the walls were replastered or smeared with cowdung by householders immediately after the application of DDT, the majority of the adults of the species rested on the under-surfaces of the roofs on which the DDT deposits were not covered up. In general it can be stated that though the resting females and males of the species can be found in the darkest corners of houses, they are not averse to resting even in the moderately lighted portions of a small hut. In Sri Lanka, however, Ariaratnam (*loc. cit.*) found that the species preferred to rest on walls below 6 feet (1.8 metres).

In villages around Delhi 77 per cent *A. culicifacies* rested on the ceiling and walls above 6 feet (1.8 metres) from the floor and hanging objects, shelves and objects on the floor served only as incidental resting places (Bhatia *et al.*, 1957). The species avoided really dark places. Cobwebs hanging from the ceiling were a favourite place of resting. These observations are more in keeping with the observations of the present author referred to above.

Generally in India, anopheline adult mosquito collections have been made in the forenoons preferably in the earlier part of the day. The use of the data collected during that period for assessing the effectiveness of indoor residual insecticides has been sometimes questioned because mosquitoes were not expected to have had sufficient contact with the insecticides. Therefore, Viswanathan *et al.* (1950) made a study in Pune District of comparative densities of *A. culicifacies, A. fluviatilis* and *A. stephensi* between 07.00 to 11.00 hours and 11.00 to 15.00 hours, in two unsprayed villages and two villages sprayed with DDT. The afternoon collections were found to be on an average about 30 per cent of the morning collections. Statistically significant positive correlation was found between them in both groups of villages and there was no indication that the two samples were not drawn from equally correlated populations. The study, however, indicated that more adults are collected in the mornings than in the afternoons.

Similar comparative observations were also made at weekly intervals in some other villages on the densities of females of *A. culicifacies* during daytime (07.00 to 11.00 hours) and at night (between 20.00 to 21.00 hours). Two villages were unsprayed, three sprayed with DDT and one with BHC. It was found that night densities were usually twice as high as in the mornings both in the unsprayed villages and those sprayed with DDT but only about 40 per cent higher than in the BHC villages. It was concluded that there was no need to resort to afternoon collection as a routine measure and that morning densities in indoor shelters gave a fair

estimate of mosquitoes populations, specially when used to assess the effect of insecticidal operations. The smaller number of *A. culicifacies* collected during afternoons even in unsprayed structures was attributed to the behaviour of the mosquitoes seeking darker or deeper places in the same houses for resting. As the day advanced their capture became difficult.

Wattal (1962) made some very interesting observations on the resting habits of *A. culicifacies* working near Delhi. He found that the numbers collected in houses were lowest in the early mornings. As the day advanced more adults could be collected at lower levels. This was attributed to the fact that as the day advanced mosquitoes moved down from the upper parts of a room to lower parts which were cooler and more attractive.

According to Pal (1945) there was no sure hibernation in Delhi during the cold weather nor aestivation in summer. He found densities of 3.4 per man hour in normal years and 25.0 per man hour during years of regional epidemics.

The gonotrophic conditions of the females resting indoors during daytime (mornings) often gives an indication of the degree of outdoor resting. If there is no outdoor resting at all the numbers or proportions of individuals which are freshly fed and half-gravid ought to be similar when the mosquitoes feed on alternate nights as is common with most species in the warmer months. There may be a slight reduction in the number of the adults because of the natural mortalities. Usually with *A. culicifacies* in South India and the Deccan the proportions are not equal. The semi-gravids are always considerably less, indicating that many adults are resting outdoors.

However, in locations where the opportunities for outdoor resting are meagre the semi-gravids may be equal or even higher than the freshly fed ones. For instance, in Eastern Afghanistan, the proportions were as follows: (Ramachandra Rao, 1951)

	A. culicifacies	*A. subpictus*
(*a*) Unfed	52	18
(*b*) Freshly fed	167	16
(*c*) Half gravid	290	46
(*d*) Gravid but with trace of blood	45	6
(*e*) Fully gravid	11	6
(*f*) Abnormal condition	5	0
	570	**92**

These observations were made in September when the average maximum temperature was about 39.3°C and the mean minimum was 14.0°C. The proportion of semi-gravids was 1.5 times the freshly fed ones indicating that outdoor resting if any was very insignificant. The locality was a highly irrigated area with few trees and shrubbery.

Host preference: *A. culicifacies* is well known to be predominantly zoophilic. Not only is this conclusion drawn from the fact that many more adults of the species are found in cattle sheds than in houses but also from the many series of precipitin tests carried out by different observers. Among the earliest studies on precipitin tests in

northern India was one by Afridi *et al.* (1939) in Delhi City. From July to early December in 1937 and 1938 they examined 8,314 blood meals in the stomachs of anophelines and found 7,249 positives for many animals. Of them the bulk had fed on cattle. The positives for human blood were:

	Total positive	Human positive
A. culicifacies	5,026	83
A. stephensi	360	5
A. subpictus	1,824	0
A. annularis	39	0

A number of other observers have also recorded low anthropophilic indices such as Barber and Rice, Pune, 1.9 per cent; Russell and Ramachandra Rao (1942c) in Pattukkottai, Thanjavur District, 2.5 per cent; Senior White and Venkat Rao (1943) 10.1 to 25.9 per cent in Visakhapatnam and Jeypore hills; Senior White (1947) 12.9 per cent in Singhbhum Hills, etc. and Shalaby (1969) 1.9 per cent in Gujarat State. However, high human blood indices have also been reported by some observers, such as Covell and Jaswant Singh (1943) in Delhi, 22.3 per cent; Russell and Jacob (1939a) in Ennore area, north of Madras, 80 per cent; Ramsay *et al.* (1936) 47 per cent in Assam and North Bengal, etc. There is apparently a wide range of variation in the anthropophily of this species.

It is interesting that in Ennore, north of Madras, a high anthropophilic index of 80 per cent was found which was obviously due to the absence or scarcity of cattle.

There is also some evidence that attraction to man changes from year to year particularly in areas liable to periodic epidemics as in the Punjab and Sind. In epidemic years biting on man increases not only because of the absolute increase in numbers but also because of metereological conditions. A relationship between blood feeding habits and prevalence of cattle was noted by Afridi *et al.* (1939) working in Delhi as the following figures show:

Ratio of man to cattle	*Anthropophilic index: per cent*
13 : 1	3.2
9 : 1	1.9
5 : 1	0.9

In Delhi in 1942 when there was an epidemic the anthropophilic index was 23.3 per cent whereas in normal years such as 1937 and 1938, the index was 1.8 and 9.93 respectively (Covell and Jaswant Singh, 1943). Pal (1945) in his observations on the bionomics of *A. culicifacies* in Delhi and its vicinity indicated that though *A. culicifacies* is not a habitual feeder on man, precipitin tests during an epidemic showed 10.1 per cent with human blood.

On the basis of these rather conflicting observations the possible existence of two biological races of this species has often been postulated. In this connection an interesting hypothesis by Roubaud (1921) may be mentioned. He postulated that the number of serrations (teeth) present on the maxillae of anopheline species determined their ability to feed on cattle or man. In order to pierce the rough and thick

skin of cattle, species with more teeth (higher maxillary index) were able to do so. Senior White (1937) determined that the maxillary index of *A. culicifacies* was 12.9. Pal (1945b) noted an index of 13.2 in specimens collected from human baited traps and 13.3 from collections in buffalo traps in Visakhapatnam. According to Roubaud (*loc. cit*) an index of 14 or less would indicate species with preference to biting on man. Other observations of maxillary index of *A. culicifacies* show that in almost all places the index has been lower than 14.0. Russell and Ramachandra Rao (1942) found no differences in the maxillary indices of *A. culicifacies* between a highly malarious area in Pattukkottai and non-malarious area in Thanjavur delta, the indices being 11.79 in the former and 11.87 in the latter. The fact that in both these areas the species predominantly fed on cattle in spite of a comparatively low maxillary index did not lend support to the hypothesis of Roubaud.

There can also be wide variations in the anthropophilic indices even within the same general area, as for instance in east central India. Senior White (1947) showed that in:

Jeypore hills	. . The anthropophilic index of females collected in houses and cattle sheds was the same.
Singhbhum hills	. . It was four times higher in houses than in cattle sheds.
Around Visakhapatnam area range	. . The anthropophilic index even in cattle sheds was high. He seemed to think that the name *culicifacies* covered a whole of cryptospecies or biological races.

Gonotrophic cycles: *A. culicifacies* is generally regarded to have a gonotrophic cycle of 72 hours after the first blood meal and 48 hours in the subsequent feeds. As in all Indian anophelines, the cycle is prolonged during cold weather.

Recently Reisen *et al.* (1976) have found near Lahore, that in *A. culicifacies* ovarian development took 40 hours inside a cattle shed where temperature ranged between 26° C to 32° C and relative humidity 78 to 100 per cent. They found multiple feedings during the same cycle in about 15 per cent of specimens.

Colonization: *A. culicifacies* has till recently been regarded as not amenable to laboratory colonization in small cages. Though Russell and Ramachandra Rao (1942a) succeeded in getting a self-perpetuating colony of *A. culicifacies* in a large (40 x 20 x 10 feet) outdoor cage they had no success in the colonization of this species in small cages in the laboratory. There has been a recent breakthrough in this. Ainsley (1976) first reported establishment of a small colony of the species in Pakistan. This was followed by similar reports by Ansari *et al.* (1977), Das (1978) and Tiwari and Reuben (1978) in India in Delhi and Salem in Tamil Nadu. The exact reasons for this recent success are not well understood. However, the recent workers have used modern techniques which were not available to the workers in the earlier years. For example Ainsley used a sophisticated system of the switches for providing artificial dawn and dusk light conditions. It was repeated by Ansari *et al.* (1977). They used cages of 72 x 60 x 60 cms. Daily at 19.00 hours four fluorescent tube lights of 40 W each were switched off and an automatic light dimmer was switched on in the laboratory. An incandescent lamp of 250 W was connected to

the automatic dimmer which reduced the voltage from 220 to 0 in 90 minutes. The process was reversed in the morning at 07.30 hours, when the tube lights of the laboratory were switched on. These arrangements were believed to simulate the dusk and dawn conditions of the field. By this method they were able to get a much better rate of insemination of females (17 to 29 per cent) whereas in the control with normal laboratory light during day and no light at night it varied from 0 to 15 per cent.

Though laboratory colonies of *A. culicifacies* not withstanding as stated above, the amenability of this species to mass rearing poses many problems. A better understanding of the biology of the species under colony condition is necessary. Das and Rajagopalan (1979) have made a few useful observations. The adults were kept in a cage of 30 cms and were given chicken blood daily and soaked raisins were provided for the males. The cages were exposed to 14 hours of fluorescent lighting. Their conclusions were:

Maximum insemination took place when the male to female ratio was 5:1. The first oviposition needed 6 days after emergence and 3 days for subsequent ovipositions. The average number of eggs laid by a female was 52 and egg hatchability 96.7 per cent. The length of immature stages was 9 days. Pupation rate ranged from 8.4 to 74.5 per cent as shown below:

	Outside room	*Inside room*
Bottom of Trays with:		
Sand	49.5	42.2
Sand and pebble	50.5	44.2
Soil from paddy fields	74.5	48.0
Red soil	27.6	14.1
Clear water	8.4	15.3

Dispersal and flight range: It had been assumed that for an effective control of malaria transmitted by *A. culicifacies* a distance of 0.8 kms would be adequate based on the impression that most of the adults of this species did not fly or disperse over longer distances. While this was in general true for most places in India it was not based on scientific grounds. As far back as 1904, James and Liston noted that in those cases where an abundant supply of hosts did not exist closeby, anophelines travelled longer distances, half a mile or more, to reach them, and an equal distance if necessary to lay eggs. However, when suitable breeding places were very near at hand they did not appear to pass them over. With special reference to *A. culicifacies* they stated that in the later part of the malaria season at Mian Mir, Pakistan, it was difficult to understand where adult *A. culicifacies* came from unless distances of over half a mile were traversed by that species. However in Fort Sandeman in Baluchistan, Pakistan, Deburca (1946) suggested that *A. culicifacies* and *A. stephensi* breeding occurred at a point 5.6 kms away from the station and was responsible for malaria transmission. James (1903) had found that no adults were found in human habitations if they were 1,200 metres away from a river and at least 540 metres from any possible source. Experience in Sri Lanka has shown that the

density was reduced by nearly 35 times if the breeding sites were cleared upto 600 to 900 metres. Some earlier records were:

Lindberg (1935)	Hyderabad, Deccan	1 km.
Bose (1934)	Birnagar, Bengal	0.8 km.
Mulligan and Baily (1936)	Quetta, Pakistan	0.8 km.
Afridi *et al.* (1938)	Kutch	Very close to habitations.

Afridi *et al.* (1940) in their experimental study carried out near Karnal, now in Haryana, released marked female mosquitoes at a common point and recaptured them at (1) that point (2) 5 stations clustered within the range of 27 to 36 metres and (3) stations at distance of 280, 300, 315 and 450 metres, all in one direction. There was some seasonal variation due to higher activity of mosquitoes from April to September than in October to December. Unfortunately the experimental set up was such that the recapture stations were all in one direction and quantitative assessent of dispersal was not possible. But the experiment showed that *A. culicifacies* females would easily disperse upto 450 metres.

In Sri Lanka, Carter (1934) found that the number of adults collected in houses fell off rapidly after 500 yards (450 metres) from a river which was the only source. His figures were:

Distance of huts from river	*Catching rate per hour*
50 — 200 yards	16.3
500 yards	9.9
1,000 to 1,300 yards	0.7
2,000 to 2,200 yards	0.5
2,500 to 3,000 yards	0.06

Russell *et al.* (1944) carried out an extensive series of experiments to determine the flight range of *A. culicifacies* in Pattukkottai area. They selected a comparatively flat open area of a radius of one mile (1.6 kms). Around a central point they constructed 80 specially designed trap-huts in four concentric circles of a quarter, half, three-fourths and one mile radius, 8 in the quarter miles, 16 in the half mile, 24 in the three-fourth mile and 32 in the one mile radius making a total of 80. The traps were distributed uniformly over the entire area. There was one small village just within the area of one mile. In each trap hut they tied a calf between 18.00 hours and 06.00 hours. The whole area was carefully patrolled. At the central point they released 54,950 *A. culicifacies* both males and females reared in the laboratory and some wild caught ones. They were marked by printer's gold dust as devised by Majid (1937). Eight batches were released between July and October 1941. They captured 207,860 *A. culicifacies* during the entire study of which 601 adults were marked ones. 558 marked specimens were recaptured in traps and the remaining 43 in the nearby villages, one of these was just within the one mile radius. 465 were captured in the traps on the first day after release and 93 on subsequent days, mostly of the second day. An analysis of these trap collections is shown in Table 25.

Table 25. Dispersal of *A. culicifacies*

Radius Mile	*No. of traps*	*No. of marked individuals*	*Percentage in each radius*	*No. of mosquitoes per trap.*
1/4	8	251	45.0	31.4
1/2	16	129	23.1	8.1
3/4	24	105	18.5	4.4
1.0	32	73	13.4	2.3
	80	**558**	**100.0**	**6.98**

Except for the concentration of the released adults in the quarter mile zone, the number of mosquitoes per trap fall down in geometric proportions in the three distal zones.

After making a statistical analysis they found that there was some factor which inhibited *A. culicifacies* adults from dispersing uniformly over the entire area though hosts for feeding were available uniformly. The inhibiting factor was distance. This obviously was related to the physical ability of the mosquitoes to fly.

The 558 specimens included 105 males and 453 females. Though males were recaptured at all distances, the percentage of marked males and females differed as shown below:

Range	*Males*	*Females*
1/4 mile	67.6	39.7
1/2	15.2	24.9
3/4	9.5	20.5
1.0	7.6	14.8

Actually eight males were collected at the distance of one mile indicating that though males could fly long distances, their average flight range was much less than that of females.

In the above study, laboratory bred and wild caught specimens were marked in powders of different colours. It was found that wild caught mosquitoes had a little longer range of flight than laboratory bred ones.

The relationship between mosquitoes captured and distance proved to be hyperbolic with an equation of the form:

$$y = \frac{a}{X^b}$$

Where
- y = expected number of mosquitoes recaptured,
- x = distance in miles travelled from the point of release,
- a = the number of mosquitoes at the 1 mile limit and
- b = measure of the flight inhibiting factor.

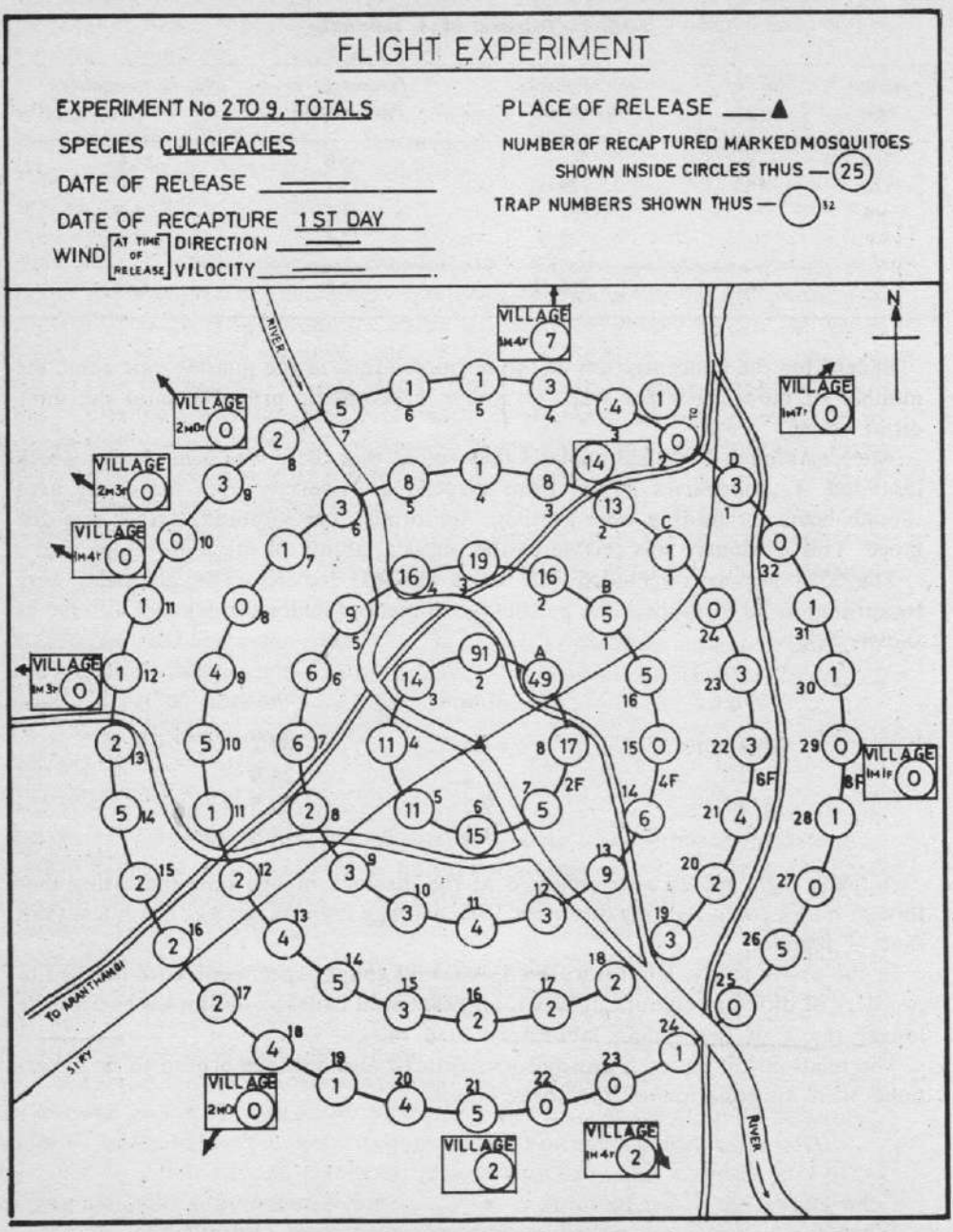


Figure 8. Flight experiments with *A. culicifacies* adults. Please see text. Eighty huts located at equal distance on four concentric circles at radii of 0.25, 0.50, 0.75 and 1.00 mile. The number collected in each hut on first day after release at a central point, in eight experiments, are shown. The distribution of the recaptured individuals was hyperbolic. (Russell *et al.*, 1944).

The *a* value is determined primarily by the number released, the *b* value measures the extent to which the flight ranges of a species group have been inhibited. The *b* value for male *culicifacies* was 1,602 and for females 0.681 indicating a higher flight inhibiting factor for males.

It can be noticed that a total of 68 per cent, or nearly two-thirds, were recaptured upto half a mile and 87 per cent three-fourth of a mile. If the inward migration of mosquitoes into a central village would also occur in the same manner and if there was a uniform distribution of breeding places, all round, it would be obvious that 68 per cent of adults in a central locality would be from distances of upto half a mile and it would be necessary to extend anti larval operations to half a mile atleast if a reduction of 68 per cent in density was needed, and upto three-fourths of a mile if a 87 per cent reduction was needed. Of course it is not clear whether the infiltration from the periphery to a central point would be of the same pattern as dispersal outwards. At any rate the average flight distance *i.e.*, the distance upto which 50 per cent of adults travel, was found to be only a little over one quarter of a mile.

In addition several marked specimens were recaptured on the first day after release in two villages at a distance of over 1.5 miles from the point of release. The total flight range recorded was between 1.5 and 1.75 miles (2.4 to 2.8 kms). Nine specimens made their flight in a single night and seven others in two nights. Three of the long flights were made against wind.

When the effect of wind direction on the dispersal was studied it was found that half the number of mosquitoes was recaptured in the quadrant against the direction of the wind and the rest were equally distributed in the other three quadrants indicating a slight but not absolute tendency to fly against the wind in the first 24 hours. Even in this quadrant more mosquitoes were concentrated at the quarter mile radius.

It is not what an individual mosquito or two can perform, but how the bulk of them behave which is important in epidemiology. The average flight distance perhaps expresses the total behaviour significant for practical purpose. Still more important is the *effective flight range i.e.*, the distance from which enough numbers fly into a village to create densities above the critical density required for malaria transmission. The effective flight distance not only depends upon the flight habits of the insect itself but also on the environment. It depends on the patterns of distribution of the breeding places, the intensity of breeding and availability and distribution of suitable hosts for feeding. If intense breeding occurs in a few breeding places even at a distance of one mile it can produce dangerous densities, in a village one mile away from them there are no hosts available in between. The manner of dispersal would certainly be different when opportunities for feeding are available closeby and when no such facilities exist. Therefore, in evaluating the role of dispersal in relation to malaria transmission one has to take both the mosquito and the environment into consideration.

In the Pattukkottai area, it has been the opinion of the present author that effective control by anti-larval measures upto a distance of three-fourth of a mile was necessary to bring down the average per man-hour densities of about 50 to 5, *i.e.*, a

reduction of 90 per cent. If the initial density in a locality is only 25 p.m.h. the reduction to 5 would require a 80 per cent reduction and if the initial density is only 10 per man hour reduction to 5 can be obtained by lowering it only 50 per cent. Therefore, distances upto which control measures would be required depend on the initial densities of the vector prevalent in a locality, its critical density and its effective flight range.

Oviposition: Like most other anophelines *A. culicifacies* can deposit eggs even in small vials in the laboratory. Recent successes with laboratory colonization also indicate that under laboratory conditions the species can lay eggs in open dishes kept in the cages. Under field conditions very few studies have been made on the ovipositing habits of this species and surveys of eggs have practically been non-existent. Russell and Ramachandra Rao (1942a) made some observations on the subject.

During their studies on swarming, mating and ovipositing behaviour of the species in a large outdoor cage, they found by direct observation that during oviposition at dusk, *A. culicifacies* gravid performed a kind of hovering movement called "the ovipositing dance". The gravid females generally hovered about 10 to 15 centimetres above the water surface and dropped the eggs on to the water when on wings. Eggs were collected directly under the hovering females by floating petri-dishes on the water surface. This observation was confirmed many times and though oviposition on the water by resting on the edge of the breeding place or on plants could not be ruled out, the performance of the dancing movement was a characteristic behaviour of the species.

Mechanical obstruction: Could this behaviour prevent the ovipositing females from laying eggs in rice fields because of mechanical obstruction provided by rice plants? When *A. culicifacies* eggs were introduced into the water of rice fields they were found to hatch normally and the larvae developed and thrived indicating that it was not the water of the rice field *per se* which was inimical to eggs and larvae.

Russell and Ramachandra Rao (1942a) carried out a series of experiments on the subject in freshly dug borrowpits of the size of about 60 x 60 cms. In the area in which they were studying, next to an irrigation canal, fresh borrowpits could be dug which would get filled with seepage water within 24 hours. The water surface was only 15 to 20 cms below ground level and observations were easy. Other studies by Russell and Ramachandra Rao (1942e) had shown that such freshly dug borrowpits were ideal places for oviposition by *A. culicifacies*.

First they determined the time of egg-laying. Three sets of borrowpits were dug and were kept covered till evening. Borrowpits were uncovered at different times of the night for the eggs to be deposited. It was found that the largest number of eggs were found in the first of the night and the second and third periods provided much smaller number of eggs.

Then in series of experiments they imposed mechanical obstructions of various kinds on the freshly dug borrowpits. They were (1) simple shading by means of dry coconut fronds providing some mechanical obstruction from the drooping leaves on the sides, (2) glass rods of the height of 3 to 4 feet were stuck into the pits.simulat-

ing the mechanical obstruction provided by rice plants and (3) living rice plants were stuck into the pits but not directly; the rice plants were stuck into the glass test tubes which were then stuck into the water. This provided a physical and living set up of rice fields but without the rice plants coming in contact with the water and preventing changes in the chemical and biological character of the water, (4) vertical bamboo strips were planted around the pits and (5) covering with wooden or wire screen boxes.

It was found that the number of eggs deposited on the water surfaces was greatly influenced by the presence of mechanical obstruction. The results are summarized below:

	No. of observations		**No. of eggs collected** (a few larvae included)	
	Experimental	*Control*	*Experimental*	*Control*
With glass rods	34	34	89	1037
Rice plants in test tubes	56	64	44	733
With surrounding barrier of bamboo strips	50	67	0	465
Shade only	19	103	548	1247

Shade by itself did not affect egg laying. In fact, against an average of 11.8 eggs in the control, 28.5 were laid in shade.

Eggs deposited in the borrow pits hatched and the larvae developed normally. The study brought out the importance of mechanical obstruction for egg laying. In the case of *A. culicifacies,* the need for space for performing the ovipositing dance was perhaps the limiting factor. Similar observations have been made by Kennedy (quoted by Bates, 1949) on a hovering dance before and during egg laying made by *Anopheles maculipennis.*

Working in Africa on *A. gambiae* Muirhead Thomson (1945) found that mechanical obstructions of various types in experimental pits did not repel *A. gambiae* from egg laying unless sedge stems were planted *around* the edges of the pit about one inch apart to form a type of obstruction similar to that provided by a thick grassy edge. The last did have some effect and the number of eggs laid was less than a quarter of that in the control. Muirhead Thompson concluded that if a single row of sedge stems around the breeding places reduced egg laying to such an extent, much greater mechanical obstruction offered by the tangled vegetation around many pools or swamps or at the water edge may deter *A. gambiae* altogether.

The practical utility of mechanical obstruction was demonstrated by Bhaskar Rao and Ramoo (1942) in Pattukkottai where they were able to control the breeding of *A. culicifacies* in channels by cultivating certain shrubs along on the banks.

Laboratory experimental studies on preferences for egg laying were made by Pal (1945). In petri-dishes kept in small cages, the number of eggs found in different types of water were: irrigation water 452, tap water 426, muddy pond water 274 and stagnant water 243.

Later larger sized cages 10 ft. x 7ft. x 6 ft. were used in each of which 50 females were released. In three experiments where the females had a choice of waters the number of eggs laid were:

I.	Soap water	0
	Tap water	397
	Pond water	169
	Muddy rain water	192
	Tank water	387
II.	Tap water	874
	Irrigation water	914
	Pond water	567
	Polluted water	348
III.	Irrigation water	492
	Pond water	302
	Tank water	272

These experiments indicated no differences in the attractiveness of the different types of waters, except that soap water was definitely non-attractive. However, such cage experiments suffer from well known limitations in that the females do not really have a chance to exercise a clear choice because of the proximity of the petri-dishes and the merging together of the "influences" of the different types of water which do not occur in purely natural conditions as in the open air.

Pal (1945) also found in laboratory experiments that the first third of the night was the preferred time for egg laying. The experiments were made as follows:

20 freshly caught gravid females were released in a cage (30 x 30 x 30 cms.) made of mosquito netting. A petri-dish with 50 ml tap water was placed inside the cage for three hours (19.00-22.00 hours). It was replaced by a second one from 22.00 to 01.00 hours and a third one from 01.00 to 04.00 hours. The number of eggs collected were:

19.00—20.00	...	1,278	
22.00—01.00	...	603	In four replications in each.
01.00—04.00	...	258	
		2,139	**eggs**

This confirmed Russell and Ramachandra Rao's (1942a) observations made fully under field conditions in southern India. Pal could have made a further very useful contribution if he had found out how many of the 20 females had really laid eggs, at each period, by counting the remaining gravid ones—a procedure not possible under field conditions. Pal found that there was no reduced fecundity during the winter months of January and February, as had been noticed in the case of *A. minimus* (Muirhead Thomson. 1941b).

Swarming and mating: Swarming and mating of *A. culicifacies* adults occur at dusk. Practically no information on this aspect on Indian anophelines was available till 1938, when Russell and Ramachandra Rao (1942a) made direct observations on the swarming and mating of *A. culicifacies* in the large outdoor cage in which many types of studies were being made. The swarming of the males could be observed against the background of the western sky just after sunset, when there was still visible light and the light intensity was about 2 foot candles. Swarming occurred for 2 to 20 minutes and females were found to enter the swarms, grab the males and fall out. The swarms were compact in size within a total volume of about one cubic foot (00.29 m^2), unlike the swarms of *A. annularis* in the open which were much larger in size. The individuals in the swarm performed a kind of floating movement both upward and downward and forward and backward. Within the cage swarming always took place on a single spot, a dry ground without any hindrance. The individuals were always found facing east.

A. culicifacies was till recently regarded as eurygamous but recent successes in the colonization in small cage show that it can be cleisto-gamous also.

Reisen and Aslamkhan (1976) have made some interesting observations in Pakistan in a village near Lahore. The observations were made in December 1975. Swarming began about 20 minutes before sunset when the light intensity was 1,414 *lux*. as against 25.4 *lux* (2 foot candles) found by Russell and Ramachandra Rao (1942a), and continued till about 20 minutes after sunset when the light intensity was only 5.4 *lux* a total period of 40 minutes as against 20 minutes found in South India. Swarms were 1 to 4 metres high and were usually formed over small features such as mounds of soil, or piles of straw, but there was little in common between the sites at which the swarms formed. The swarms were principally composed of males. The females entered the swarms and pairing was observed from 6 minutes before until 16 minutes after sunset. The pairs which copulated, on an average 27 seconds, left the swarms and drifted slowly to the ground. All the females which came to mate were nulliparous but 7.8 per cent had taken a partial blood meal on the same or the previous night. Fully engorged females were rarely collected in the swarms. The few differences from the observations in southern India could be due to the long twilight at the northern latitudes and the fact that the observation in the Punjab were quite in the open while in southern India they were made inside a cage even though a large one.

Biting time and activity: There has been much disagreement among workers regarding the time of biting by *A. culicifacies*. It is however now regarded to be a night biter by almost all workers and some very early observations made by workers like Clyde (1931) and King and Krishnan (1929) that the adults bite during daytime as well as at night have not been repeated. Many discrepancies in observations appear to be due to differences in methods but a few could really represent natural variations. Nursing *et al.* (1934) found in the Malnad area of Karnataka that 67 per cent of *A. culicifacies* and *A. fluviatilis* was found in a tent between 21.00 and 23.00 hours were two and a half times more than those made between 03.00 and 05.00 hours. As Muirhead Thomson (1951) has stated, it is difficult to

state whether they really represented biting activity. Afridi and Puri (1940) in Delhi and Senior White (1938) in east central India found that the main biting activity occurred around midnight, which was confirmed by Pal (1945) i.e., between 20.30 and 01.00 hours. However, Senior White (1946) found in Hazaribagh in central India, that the night prevalence gradually decreased from 23.00 hours upto 05.00 hours supporting the view that the period of highest activity was before 23.00 hours. There was the usual increased entry at dawn.

Sustained and critical observations made by Viswanathan, Ramachandra Rao and Halgeri (1955) in specially constructed huts in a rural area near Pune between December 1951 to May 1952 have thrown much light on the subject. 13 species of anophelines were collected but only data for *A. culicifacies* were analysed. The huts were 8 feet x 6 feet were made of thick bamboo matting. They had a gabled roof the highest point of which was seven and half feet from the ground. There was one door, 1.5 x 0.6 metres, at one end. If necessary, the door could be closed tightly. There was a window with an outlet window trap on one side. At the middle height of the two long sides there was a long longitudinal opening in which the upper part overlapped the lower part to provide an easy entry but not a suitable exit. The wall was plastered with mud inside and the roof was lined by fine matting so that the entire internal surface could be easily searched. There were abundant breeding places for *A. culicifacies* nearby. A buffalo was tethered in the huts each night between 18.00 and 07.00 hours.

It was found that more mosquitoes entered the huts when the door was kept open than when only the lateral slit entry was available. In 54 observation nights, an average of 0.8 males and 8.3 females were collected per night when the door was kept closed and 11.7 and 41.3 respectively when the door was kept open. The huts were thoroughly searched and collections to depletion made every evening between 17.30 and 18.00 hours so that no mosquitoes remained inside before the all-night observations commenced. Again this was done between 07.00 and 07.30 hours. Collections to depletion were made for half-an-hour every three hours by two highly trained mosquito collectors i.e. between 20.30-21.00 hours, 23.30 hours—midnight, 02.30-03.00 hours and, 05.30-06.00 hours. During 81 nights, the numbers collected were as follows:

	Door closed		**Door open**	
	No.	*Per cent*	*No.*	*Per cent*
1st quarter	329	74	490	44
2nd quarter	71	16	254	23
3rd quarter	9	2	83	7
4th quarter	19	4	151	14
07.00-07.30	19	4	140	12
	447		**1,118**	

Ninety per cent of *A. culicifacies* females entered the hut before midnight when the door was closed and 67 per cent when it was open, and the first quarter provided 74 per cent and 44 per cent respectively.

Studies on the gonotrophic conditions of the females entering the huts using the following classification were made (See also Fig. 1 and Chapter 4).

Some semigravid and fully gravid specimens (15%) also entered the huts at night, indicating some degree of movement of females throughout the night, not necessarily associated with feeding or oviposition. They must have been resting outdoors or in other shelters during night. It was observed that among the freshly fed specimens there were 7.1 per cent which had taken a second blood meal in the middle of the gonotrophic cycle and 12.3 per cent had taken only partial meals. The epidemiological significance of repeated blood meals is obvious. It was observed that a few females and males entered the hut between 07.00 and 07.30 hours, i.e., soon after sunrise. They were obviously shelter seekers. The outlet window trap was unsatisfactory as in over 120 nights of observations only 33 adults were collected (16 males and 17 females).

No conclusion regarding the degree of outdoor resting was possible. When a hut was sprayed with DDT to study the behaviour of *A. culicifacies* in sprayed structures it was found that DDT spraying offered no bar to entry and taking of a blood meal. But the adults were killed, but no excito-repellent effect was noticed.

In Lahore, Reisen *et al.* (1976a and 1977) have made critical observations on several aspects of the bionomics of *A. culicifacies*, particularly on the crepuscular and nocturnal activities. Reisen *et al.* (*loc. cit*) first found the mean number bites on buffaloes at various times of night for 15 minutes each period.

18.45-19.00—0.3
20.00-20.15—1.3
23.00-01.15—2.7
02.00-04.15—2.6
05.00-05.30—0.6

These studies were made in August 1975 during the monsoon months.

Still later Reisen and Aslamkhan (1978) found *A. culicifacies* extremely abundant throughout, including in winter months, though that marked changes occurred in its feeding times. During November to January, i.e., in the colder months, most biting occurred outdoors between dusk and 20.30 hours. The observation was similar to that by Viswanathan, Ramachandra Rao and Halgeri (1955). However, during April, May, September and October most biting shifted to the period between 21.00 hours and 01.00 hours while in mid-summer i.e., June, July and August the biting showed no rhythm and equal numbers were collected throughout the night. This seasonal variability according to them, could be responsible for the conflicting reports about the biting habits of this species recorded by different workers. Reisen *et al.* (1977) have described a similar shift in the time of swarming and mating in *A. culicifacies* which was also indirectly correlated with temperature. The seasonal shifts did not appear to be related to the movements of potential hosts, *i.e.*, cattle

indoors or outdoors. For example, in March the crepuscular habit predominated although all hosts remained outdoors throughout the night. Therefore, season and climate could be important factors in determining the time of entry and feeding.

Longevity: Using wild caught mosquitoes from cattle sheds, Afridi *et al.* (1940) released 74,226 *A. culicifacies* at a central station in 93 batches between August 1936 and November 1938. In the earlier period, prior to November 1936, they were marked with printers dust of the same colour; subsequently different colours were used for different batches. They recaptured 1,570 specimens. After discussing the limitations of the methods used, including the fact that the released specimens were of uncertain age, because they were wild caught, they analysed the data by carefully correlating the field data with the probable length of life as determined by Perry's wing grades. In 43 batches in which the wing grade II contributed to 80 per cent of all mosquitoes the average longevity was 8.5 days. In 36 batches in which there was a high proportion of wing grade III (older mosquitoes) the average longevity was 14.0 days. It appeared that the increased longevity was associated with season. Both in field and laboratory studies the longevity figures were higher in the autumn than in the rainy season. In April and May, however, laboratory mosquitoes lived much longer than the field mosquitoes, obviously because of the better protection afforded by laboratory conditions.

Laboratory studies carried out in Lahore in 1940-41 by Pal (1943) provided some interesting information on longevity. Females exposed to constant temperature of 40°C did not survive for more than 24 hours, irrespective of changes in the relative humidity. However, at 35°C, between relative humidities 20 to 100 per cent, they lived from 4 to 10 days; very low or very high relative humidities at these temperature were not favourable for the life of these species. At 30°C they survived from 6 to 18 days and at 25°C from 14 to 28 days. The relative humidity of 60 to 80 per cent seemed to be the most favourable. At low temperatures between 12°C to 18°C survival was extended to 4 to 8 weeks. Temperatures below freezing point (minimum 2°C) were lethal to the adults after 5 minutes of exposure. 41°C was considered the thermal death point for the adults. Field observations (Pal, 1945) generally conformed to those made in the laboratory. Seasons with the temperature between 25°C and 30°C and relative humidity of 60 to 80 per cent appeared to represent the most favourable climatic conditions, which occur in most parts of India during September, October and November. The species was found to overwinter both in larval and adult stages in the Punjab.

However, it should be noted that while laboratory studies can give very useful indications as to what happens in nature, direct studies under natural conditions can alone provide information of relevance to epidemiology. Such direct observations are difficult and require elaborate studies.

Indirect observations can be made in several ways. For example, Covell and Baily (1930) found that in the warm months of March-April, in Sind, there were many specimens with oocysts but none with sporozoites, indicating that the adults of *A. culicifacies* died before reaching the dangerous age. Macdonald and Majid (1931) made similar observations in Punjab.

Perry's wing grades, as already stated, can also be used but the most sensitive is the use of Detinova's techniques for counting the numbers of ovipositions by examining the number of ovarian dilatations. All these methods have been used with varying utility.

An elaborate study, in conditions nearly simulating nature, was undertaken by Russell and Ramachandra Rao (1942b) in Thanjavur District of Tamil Nadu in the year 1941. A large outdoor cage measuring 40 x 20 x 10 feed was constructed out of 16-mesh aluminium screen, which allowed ample light and ventilation. The entry into the cage was through a double door which prevented any mosquito from going out or coming in. In one part of the insectary was a small mud and thatch hut measuring 10 x 8 x 8 feet. It had an incomplete partition of 6 feet height in the middle, separating the two chambers in one of which a calf was tied each night between 18.00 and 06.00 hours and metereological instruments were kept in the other. The roof of the hut was thatched but inside it was lined by blue cloth. Hanging from the roof were three pieces of cloth of the same material dividing the gable portion into three dark compartments, attractive to the mosquitoes as daytime resting places. The entire structure was such that the whole interior could be searched very thoroughly for resting mosquitoes.

Apart from the calf which provided a source of blood meal for the females, glucose solution on cotton pads and soaked raisins were offered for the males in petridishes. The insectary also contained a typical domestic well and several borrow pits filled with fresh seepage water, which were highly suitable for *A. culicifacies* egg laying. A few small flowering plants such as Cosmos, Zinnia, Vinca and Thevesia and a few banana plants and a small portia plant were also grown inside the cage. In brief, the interior of the cage simulated a small farm house with its surroundings. Except for the restriction of long flights, the cage provided a complete environment for resting, feeding, swarming and mating and egg laying for adults, and ample opportunities for breeding in ideal types of breeding places.

Batches of laboratory bred adults were released inside the cage at frequent intervals. Those used in connection with longevity studies were marked by printer's gold dust by the technique of Majid (1937). When two or more batches were being observed simultaneously, distinctive colours were used. At frequent intervals the insectary was very thoroughly searched and the mosquitoes were captured as gently as possible directly and individually into small test tubes. No suction tubes or nets were used. The number of marked mosquitoes was carefully noted and they were released back immediately. Only laboratory bred specimens which had hatched out fresh the night before the release were used. The exact age of the mosquitoes was known. In all, 16 batches were released between April to October 1941. The numbers released per batch varied from 100 to 1,793. The total number released during the entire study were 4,499 males and 5,913 females (total 10,412).

The maximum longevity noted among the several batches and an indication of the temperature and humidity conditions are shown in Table 26.

Table 26. Longevity of *A. culicifacies* in seminatural conditions

	No. of batches	*Longevity' maximum number of days*	16.00 *Hours* temperature Range °C*	16.00 *Hours R. H. % Range*
April & May	4	8 to 11	30.5—34.9	57.1—63.5
June	1	13 to 14	30.4—32.0	61.8—71.6
July	3	6 to 24	31.0—31.2	71.5—81.6
August	3	21 to 23	30.9—31.5	78.3—83.3
September	2	23 to 34	29.2—30.4	78.3—83.8
October	3	25 to 32	28.0—30.0	79.6—88.0
November	—	—	25.6—28.1	77.3—87.2

*The 16.00 hours figures have been used as it has been found that the temperature and R. H. at that time give a better indication of the daily means than those at 08.00 hours.

The data indicated that the total length of life gradually increased as metereological conditions became more favourable.

With special reference to eight batches in which adequate numbers were used, particularly batches 9 to 16 released in August to October, the data were further analysed. It was observed that the mortality occurred at the rate of approximately 50 per cent every two days. Macdonald, who had re-examined the data, stated that the mortality could be better expressed as about 24 per cent each day.

Plotted on logarithmic scale, the mortality was found to be in a straight line (Fig. 9). The average longevity was 4 days and the probable expectation of life (which is the expectation of life of an individual at any given moment) was two days. The maximum length of life of males was eight days only and of females 34 days. From an epidemiological point of view, the most significant aspect of longevity is the number and proportion of individuals of the population of females lived longer than 10 days. Therefore, inspite of the low longevity of the majority of individuals there would be enough old individuals to carry on malaria transmission. The higher the intial number the higher the number of individuals which live longer than ten days. They also noted a tendency of the mosquitoes which survive longer than 10 days to live longer than the average.

Russell and Ramachandra Rao compared their data with those of Afridi *et al.* (1940) in the Punjab, collected by quite different methods. Though the climates of the Punjab and Thanjavur District do not exactly correspond, the maximum longevities revealed a similar pattern (Table 27).

Regarding longevity, a difference exists between the observations of Russell and Ramachandra Rao (1942a) in Pattukottai and of Brooke Worth (1953) in the Malnad of Karnataka. The former workers found the shortest survival time during May and June when the temperature was high and humidity low. Brooke Worth feels that a different pattern would have been found if they had kept the mosquitoes in an unoccupied cowshed because the species characteristically rests in such shelters

during day time. It should, however, be noted that Russell and Ramachandra Rao's studies were designed to observe the conditions as they existed in as natural a condition as possible. The studies were made to observe the effect of macroclimate rather than the microclimate and to determine the gross rates of survival. Further the hut inside the cage was to all purposes a natural cowshed. It cannot be doubted that if the adults had been kept in small cages within a cool hut or cattle shed they would have shown a different pattern of longevity.

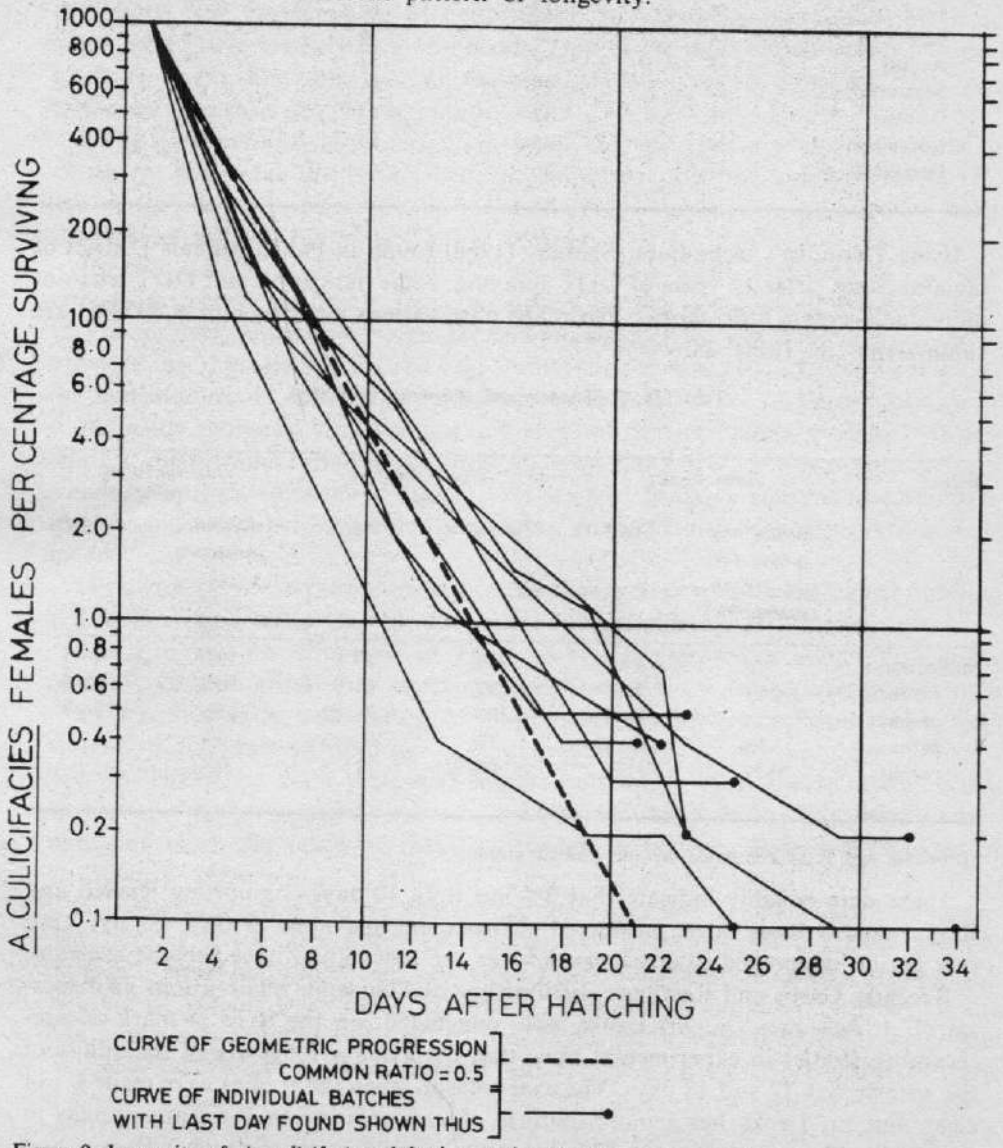


Figure 9. Longevity of *A. culicifacies* adults in a series of eight experiments in a large outdoor cage. (Russell and Ramachandra Rao, 1942b)

Table 27. Longevity of *A. culicifacies* in Punjab and Tamil Nadu

	Afridi *et al.* (1940)		Russell & Ramachandra Rao (1942)	
	% R.H. at 16.00 hours	*Maximum length of life*	*% R.H. at 16.00 hours*	*Maximum length of life*
May	24	9	61	11
June	56	14	65	14
July	64	24	78	24
August	48	14	81	34
September	52	25	81	23
October	36	32	83	32
November	32	45	84	—
December	47	56		

Using Detinova's techniques, Shalaby (1968) found in Panch Mahals District of Gujarat State, after 12 years of DDT spraying, some indication that DDT resistant individuals were a little shorter lived. The observations made by him in 1959-61 are summarized in Table 28.

Table 28. Resistance and longevity (Shalaby)

Season	*June-August 1959*		*January-May 1960*		*June-October 1961*	
	Before any sprays for the season (susceptible)	*Age**	*Tolerance*	*Age**	*Definite resistance*	*Age**
Nulliparous	34%	4—5	30%	5—6	19%	4—5
1 - parous	56%	5—7	59%	6—9	76%	5—7
2 - parous	7%	7—9	10%	8—10	5%	7—9
3 - parous	3%	9—11	1%	10—12	0%	
4 - parous	0.2%	11+	0%	12+	0%	

*Probable age is as computed by the present author.

These data roughly indicate that 3% live upto 10 days (supporting Russell and Ramachandra Rao) in an unsprayed environment and none or only 1% live upto that period in sprayed situations even when the mosquitoes have become resistant.

Recently Curtis and Rawlings (1980), while making some observations on dispersal of *A. culicifacies* in Sri Lanka have concluded, on the basis of mark-release-recapture studies in experimental huts, that the average longevity of the adults of the species was 17 and 18 days. The exact season when the studies were made is not clear, but Sri Lanka has a more uniform climate than north India and similar to that of the Thanjavur District. This longevity is much more than what Russell and Ramachandra Rao estimated, but even these authors had found maximum longev-

ity of 24 to 32 days during the irrigation season. The studies of Curtis and Rawlings are continuing and the results of further studies would have to be awaited.

The effect of microclimate, however, is important. In a few observations made by the present author in a rural area near Pune, a sporozoite infected *A. culicifacies* female was found during May, at a time when the maximum temperature of the air in shade ranged between 40°C and 42°C. This would never have been possible unless the adult had regularly found a sheltered niche ideal in every respect.

Density: The measurement of densities of *A. culicifacies* mosquitoes has been made by malaria workers in many places. One of the most common and practical methods is the determination of the numbers collected per unit of time, *i.e.*, expressed generally as per man-hour.

While this method has limitations when the densities are extremely low or extremely high and the effort made in collection does not produce reasonably related figures of prevalence, it has been of practical utility for the use of research workers as well as of public health workers.

The per man-hour figures provides a very useful method of assessing relative prevalences between different areas or between seasons in the same area. It has been very extensively used in this country. An example of the utility of this method is provided by the work of Russell and Ramachandra Rao (1942c). They compared the densities of *A. culicifacies* in the newly irrigated areas of Pattukkottai with those in the thousand-year old irrigated areas of the old Thanjavur delta. Malaria was prevalent in the former and almost nil in the latter area. In a two-year study, in which collections were made regularly in selected catching stations and standard trap-huts in a number of villages in both the areas the following observations were made:

1. *A. culicifacies*, occurred in both areas. There were no morphological differences in the eggs, larvae and pupae and adults occurring in the two areas.
2. There was no difference in susceptibility to plasmodial infections because adults from both areas were about equally infected when fed on common donors.
3. Densities of *A. culicifacies*, however, showed different patterns which were related to the then existing agricultural practices.
4. In Thanjavur delta there was intense cultivation of ricefields in which two crops were regularly taken between June and February. In the Pattukkottai area only one crop was taken at that time, generally between June and October or October to February.
5. In the delta there was no waste irrigation water as every field was fully exploited, but in the latter area only a small proportion of the irrigable areas was actually irrigated. There was abundance of waste irrigation water flooding all sorts of fields. Because of the design of the canal system, the entire water supply needed for the whole area was released at once, and therefore a lot of water which did not irrigate ricefields turned into waste irrigation water, filling up many uncultivated fields, borrowpits and ditches, etc. In fact there was more waste irrigation water than water on fields with crops. In the old delta there was absolutely no waste irrigation water.

6. This resulted in enormous breeding of *A. culicifacies* and in high prevalence of *A. culicifacies* adults in Pattukkottai while the densities of the species were continnually below 8-10 per man-hour in the Thanjavur delta with a small short spurt for less than two weeks in the month of June when fresh irrigation water was let in. In the Pattukkottai area, however, very low densities well below 5.0 per man-hour prevailed during the non-irrigation season from January to May but the densities went up to high levels, sometimes upto 50 per man-hour or more between June and October and remained so for 8 to 10 weeks. With the termination of the irrigation season the density came down.

7. Similar differences in densities were also found in standard trap-huts constructed in the observation villages in both the areas confirming the trends of per man-hour figures.

8. Based on the above observations it was concluded that the high malaria prevalence in the Pattukkottai area was due to high densities of *A. culicifacies* above 7 to 15 per man-hour during the season reaching upto 50 per man-hour continuously for several weeks. For practical purposes, and for safety, 5 per man-hour was regarded as the density below which malaria transmission would not take place.

It was presumed that when irrigation became as intense in Pattukkottai as in the old delta and there was no waste irrigation water, the Pattukkottai area would also become free from malaria. This did come about soon after, because of the intense rice cultivation from 1942 onwards encouraged by the government, due to the stoppage of rice imports from Burma. Today the malaria potential in the Pattukkottai Taluk is very low.

The above studies lent support to the hypothesis of Ross (1910) that there was a density of the vectors (critical density) below which malaria transmission would cease. The hypothesis has also been further tested and enlarged (Macdonald, 1957).

Larval Ecology

A. culicifacies is a species which has been extensively studied regarding its breeding habits. The larvae have been found in a variety of breeding places, but usually in water which is not rich in organic matter. Larvae have been found in irrigation channels, swamps, river bed pools, tanks and ponds, in certain stages of rice fields, irrigation wells and even sometimes in brackish waters. It has been known to breed prolifically in rain water accumulated in sandy deserts of Western India and in Pakistan. In fact it is an ubiquitous species and is found in all types of breeding places except perhaps tree holes, leaf-axils and man made cement or iron cisterns. It has been found in hoofmarks, cart-tracks and underground 'karezes' (Underground water supply aqueducts) in Pakistan.

The literature on the subject being too extensive to permit detailed references the present review will deal largely with the important studies done more recently.

In an extensive study on the anophelines of Pattukkottai, Russell and Ramachandra Rao (1940a and 1941a) made many observations on the breeding habits of the species. In a detailed analysis of over 6,033 larval searches of which 5,166 were

positive for larvae, they found that *A. culicifacies* was found in 56.8 per cent of all positive searches (3,190) and the number of larvae of *A. culicifacies* collected was 49,129 i.e., 34.8 per cent of 141,119 larvae of all species. *A. culicifacies* larvae were found in the following breeding places in the period of the study (Table 29a):

Table 29a. Breeding places of *A. culicifacies* in Pattukkottai.

Breeding places	Number of searches	Number of times *A. culicifacies* found	Number of *A. culicifacies* larvae found	Number of larvae of all species found
Irrigation channels	767	645	10,884	13,213
Wells (all types)	1,240	623	12,377	23,264
Field channels	507	378	4,890	7,779
Waste irrigation water	668	467	8,041	14,995
Borrowpits	396	270	4,846	10,445
Tanks	1,523	461	3,754	23,269
Ditches	299	217	3,520	6,691
Seepage and spring pools	173	123	2,541	5,200
Rice fields (fallow)	336	184	1,717	7,057
Rain water pools	124	64	1,263	8,423
Pool, hoof-mark and cart-track	75	56	1,012	1,769
Rice field growing	779	115	758	10,558
Total	**6,035**	**3,014***	**44,786***	**127,552**

*These totals are not in agreement with the numbers given in the text because of exclusion of certain minor breeding places and discrepancies.

The number of times *A. culicifacies* was taken in different habitats expressed as percentage of total times the species was taken in all places, the number of larvae taken in a given breeding place expressed as a percentage of larvae of *A. culicifacies* taken in all places are given in Table 29b.

Table 29b. Proportions of *A. culicifacies* larvae in breeding places in Pattukkottai

Breeding places	A	B	C	D
Wells	20.7	27.6	50.2	32.5
Tanks	15.3	8.4	30.3	16.1
Rice fields (growing)	3.6	1.7	14.8	4.6
Waste irrigation water	15.5	18.0	69.9	53.6
Irrigation channel, etc.	21.4	24.3	84.1	82.4
Borrowpits	9.0	10.8	68.2	46.2
Field channels	13.2	10.9	78.5	62.9
Rice fields (fallow)	6.1	3.8	54.8	24.1
Ditches	7.2	7.9	72.6	52.6
Seepage and spring pools	4.1	5.7	71.1	48.9
Rain water pools	2.1	2.8	51.6	36.9
Hoof-marks and cart-track	1.9	2.3	74.7	57.2
All places	50.0	35.1	50.0	35.1

A — Number of times *culicifacies* larvae were taken in a given type of breeding place expressed as a percentage of total times and the species was taken in all places.

B — Number of larvae of this species collected in a given type of breeding place expressed as percentage of total larvae of the species taken in all places.

C — Percentage of times the species was taken in all visits to the particular breeding place.

D — Number of larvae of the species expressed as percentage of the total larvae of all species collected in the particular breeding place.

The totals of percentages in columns A and B of Table 29b do not add up to 100 because of duplication of some figures such as under borrowpits and waste irrigation water.

The above data bring out the fact that irrigation channels, field channels, waste irrigation water and hoof-marks and cart-tracks were most preferred breeding places of this species. The least preferred were growing rice fields and tanks.

A. culicifacies was found alone in 31.3 per cent of breeding places while *A. stephensi* was found alone 56.8 per cent and *A. nigerrimus* 34.0 per cent. It should be noted that *A. stephensi* was most fastidious being taken only in wells.

A. culicifacies was most frequently associated with *A. subpictus* because they had very similar habits.

Russell and Ramachandra Rao noted that relatively large numbers of wells and tanks were searched because in all the summer months there was no irrigation water, field canals or borrow pits and other places. 59.0 per cent out of 263 larvae positive wells searched contained *A. culicifacies* larvae (Total 12,377). Irrigation wells seemed to be more favourable, this species being found in 55.5 per cent of such wells, while only 28.2 per cent of domestic wells yielded *A. culicifacies* larvae. Moreover the larvae in wells were more numerous per unit of surface area than any other place except river bed pools. Of course, in wells the most predominant species was *A. varuna,* 16,973 larvae having been found in 623 wells.

Among the minor and miscellaneous breeding places, *A. culicifacies* larvae have been found in pools in the canals, river-bed pools, seaside marsh, turf-pool, river-edges and artificial container (taken only twice).

During the above study, detailed information was kept on such features of the breeding places as salt water, brackish water, no shade or partial shade or dense shade; clear or turbid or very turbid water, stagnant or sluggish current; vegetation absent, scanty or abundant; presence of algae, blue green algae, floating higher vegetation, emergent higher vegetation and submerged vegetation, etc. In a further analysis (unpublished) of data the present author found that ecologically the most ideal breeding places and the most unsuitable breeding places had the following characteristics:

Most suitable — Clear or only slightly turbid water, not brackish, either stagnant or with a slight flow without shade and without growths of any vegetation or macroscopic algae.

Least suitable — Highly turbid, stagnant water, highly brackish, deeply shaded and with good growths of vegetation, including floating, submerged or vertical vegetation, growths of blue-green algae and rich plankton.

Between the two extremes were a wide range of variations. Among the intermediate types the one found to be most common was a clear or very slightly turbid water, with a perceptible flow and grassy margins without growth of blue-green algae.

The first type is found in river bed pools and freshly excavated borrowpits. The second is exemplified by tanks and ponds, marshy rice fields, borrowpits or ditches with gross organic contamination. The intermediate type was found in field channels with flowing water.

It was noted in one experiment that when irrigation wells, very suitable for *A. culicifacies* larvae, were contaminated by cut pieces of cactus thrown into them the breeding of *A. culicifacies* suddenly stopped. The effect of cut vegetation perhaps resulted in increasing organic matter (nitrogenous matter).

Observations, though of not such a comprehensive nature made in other areas of India have shown more or less similar breeding habits. In the Ennore-Nellore areas north of Madras, the species was very prolific in shallow pits dug in casuarina plantations (Russell and Jacob, 1939a). Barber and Rice (1938) in Pune found the breeding in wells during the hot and dry weather when other breeding grounds were absent. Studies in Northern India and Pakistan by workers such as McCombie Young and Majid (1930), Ramsay and MacDonald (1936), Mulligan and Baily (1936), Deburca (1946), etc. have found very similar habits. The association of *A. culicifacies* breeding with irrigation water and rain water has been well established all over India.

Covell (1938) also showed that *A. culicifacies* could breed in artificial collections of water such as ornamental waters and unused swimming pools. The importance of rain filled borrowpits dug on the sides of roads and railways has been most amply established.

In Sri Lanka, Rajendram and Jayawickreme (1951) found that though *A. culicifacies* preferred to breed in ground water, free of vegetation, shade from the trees was not favourable. The proportions of larvae found in shade was only roughly 1/10 of those in the sun in pools. In streams the proportion was only 1/3.

With particular reference to rice fields, the work of Russell and H.R. Rao (1940) is very interesting. They made a series of extensive observations on *Anopheles* breeding in fields in Pattukkottai 10 out of 12 species occurring in the area were found in rice fields the species absent being *A. aconitus* and *A. varuna*. There was strong evidence to suggest that distribution and density of the species in rice fields depended on the stage of rice growth than the season of the year. Anopheline species in the rice fields had the following pattern of succession and association:

1. Fields freshly watered but not ploughed (in the order of occurrence): *A. culicifacies* followed by *A. subpictus* and *A. pallidus*.

2. Fallow fields flooded and ploughed (very turbid water): *A. subpictus, A. culicifacies* but *A. culicifacies* scarce.
3. Growing rice fields, first stage, plants less than 12 inches high: *A. pallidus* and *A. culicifacies* in about equal densities, but rapidly *A. pallidus* increased and *A. culicifacies* decreased.
4. Growing rice fields, second stage, after rice plants were 12 inches high: *A. pallidus* underwent a progressive decrease and *A. nigerrimus* progressively increased. *A. culicifacies* became very scarce.

If the number of all species of *Anopheles* larvae were taken together, larval density was higher in the newly transplanted fields but it fell progressively as plants became taller. An interesting relationship of the presence of algae and the breeding of *A. culicifacies* was noticed. The species was most abundant when no macroscopic algae were seen in the fields and minimal when floating masses of both green and blue-green algae were present.

Russell and H.R. Rao (*loc. cit.*) by placing suitably designed mosquito nets over selected spots on the rice fields, made an estimate of the probable output of adults. For every 100 larvae estimated to be present the number which grew into adults ranged between 4.3 and 7.6 per cent. Experiments on intermittant irrigation of fields indicated that very good control of breeding of anophelines was obtained by weekly cycles of two dry days and five wet days, when rain did not interfere with the drying.

The ecology of *A. culicifacies* larvae in seepage waters in borrowpits which were ideal breeding places was studied in some detail under field conditions in Pattukkottai by Russell and Ramachandra Rao (1942d) 68 per cent of all borrowpits examined contained larvae of this species and 46 per cent of all larvae taken from borrowpits belonged to this species. Breeding in the borrowpits gradually declined as the season advanced. A study was, therefore, designed to understand the factors involved. New borrowpits were specially dug on a piece of land adjacent to an irrigation canal. They soon became filled with seepage water and eggs were seen to be deposited from the very day the pits got filled. Some borrowpits were dug during each month from June 1938 to January 1939 and almost daily observations were made. Eggs were regularly collected in a few selected borrowpits every day by using wire loops. Apart from the collections of eggs and larvae, several chemical, biological and physical studies were also made. The results can be summarized as follows:

1. In seepage filled borrowpits there was a progressive decline in the density of larvae of *A. culicifacies*, as the pits became older. The largest number of eggs and larvae were found soon after water entered the newly dug pits.

2. There was less ovipositing by *A. culicifacies* in older pits than in new ones dug late in the irrigation season. Newer pits seemed definitely more attractive to the species than older ones. The newer pits sheltered more *A. culicifacies* larvae late in the season than the older pits.

3. The decline of *A. culicifacies* larvae density in a borrowpit seemed to be due mainly to factors internal to the pits. There was no evidence of the influence of

external factors, except from October to January when perhaps meteorological influences supplemented the internal factors. The attractiveness of new borrowpits to *A. culicifacies* appeared to be due mainly to internal factors.

4. Certain common factors studied did not seem to have any significance in relation to *A. culicifacies* density in the pits. Rainfall, predators, macroscopic vegetation, pH, CO_2, hardness, chlorine, ammoniacal nitrogen, nitrates, nitrites, sulphates and iron appeared to have no significance in this regard. Albuminoid nitrogen and oxygen absorbed, perhaps, had some significance, which was not clear.

5. Among planktonic organisms, the individual groups of organisms, such as unicellular green algae, diatoms, rotifers and copepads definitely showed no relation to *A. culicifacies* breeding. Protozoa as a group appeared to be negatively associated to a slight degree. Blue green algae also seemed to have a definite negative association.

6. Amorphous organic matter and total plankton, however, showed statistically significant negative association with larval density of *A. culicifacies*. The decline in *A. culicifacies* larvae was clearly associated with increase in total plankton and amorphous matter. The attractiveness of the new borrowpits also seemed to be related to their low plankton content.

7. The exact manner in which the total organic matter acted as an inhibitory factors against *A. culicifacies* breeding was not determined.

Microscopic plankton was studied in detail with special reference to green and blue-green algae, diatoms, protozoa, rotifers, copepods, amorphous matter and total plankton. Standard techniques of measurement, *viz.*, Sedgwick Rafter funnel and Whipple counting cell and disc, were employed for quantifying the plankton. In all, 19 pits were kept continuously under observation during the season.

An auxillary study of the number of eggs laid by *A. culicifacies* females in different seasons provided the data shown in Table 30.

Table 30. Average number of eggs laid by wild caught *A. culicifacies* at different seasons (1940-41)

Months	*No. of females*	*Average number of eggs laid*
February—March	6	131
April—May	12	111
June—July	14	135
August—September	50	105
October—November	56	115
December—January	25	123

No difference in the average number of eggs laid in different seasons could be seen.

Among the physical characters the temperature of water was the most important. Air temperature variations in that area were not marked. For instance, the temperature in October was not more than 2° C below those in July and August and in November and December it was only 3° C below that of October. In no year of observation had the temperature difference between higher and the lower monthly

mean been more than 6.3°C. The least monthly mean observation was 24.2°C in December and the highest monthly mean was 30.5°C in May and June. Relative humidity was usually below 60 per cent. This would indicate that temperature by itself could have had very little influence on egg-laying during the period of study.

Effect of temperature (Laboratory studies): Pal (1945) made a series of studies on the temperature conditions which influence egg laying and development of larvae. He noted that the only previous observations on temperature as a factor were two, viz. one by Senior White (1928) who recorded 28°C as the optimum temperature for development of larvae and second by Muirhead Thomson (1942c) who recorded that 44°C was the maximum temperature (or the thermal death point) for the survival of the species. Pal used water baths with different temperatures and came to the conclusion that:

(*a*) Ovipositing females showed no selective preference to warm water (38°C) and hot water (42°C) but when a choice of warm water (38°C) and very hot water (45°C) was offered, the females preferred the lower temperature.

(*b*) The most favourable range of temperature for viability of eggs was 28°C to 36°C. Both lower and higher temperatures, 20°C and 40°C, were unfavourable for development of eggs. For larval growth temperatures of 32°C to 37°C were most favourable taking 11-15 days and an emergence rate of 60 per cent. But the range of 28°C to 32°C was the most suited for eggs, larvae and pupae.

(*c*) The thermal death point for eggs was 53°C though even with 50°C only 12 per cent of eggs would hatch. For full grown larvae 45.5°C was the thermal death point and for pupae 43.3°C.

(*d*) In several types of waters in the field the maximum temperature recorded in the hot summer months was 44.7°C, but commonly it was 43°C. However, in these months large bodies of water such as tanks rarely had temperature above 37°C and they could provide shelter for the species during adverse months.

(*e*) The lowest temperature tolerated by eggs was 5°C, larvae 3°C and pupae 3°C. As the temperatures of natural waters of breeding places in the Punjab during winter are generally not lower than 8°C, uninterrupted breeding could be expected to occur throughout the winter months, though at a lower speed of growth.

Other studies: In Sri Lanka the heaviest breeding takes place in the pools of the rivers and streams and the species occurs in negligible numbers in all other breeding places, usually found in villages (Rajendram and Jayawickreme, 1951). The high prevalence of *A. culicifacies* is associated with drought conditions. Due to failure of the monsoon numerous pools are formed in the river beds providing ideal breeding places for the species. The prevalence of *A. culicifacies*, however, varied between the wet zone, the dry zone and the intermediate zone into which the island could be divided epidemiologically. In Anuradhapura town the peak season was November,

December and January coinciding with onset of the northeast monsoon. (Also Tyssel Jones, 1951). The great epidemics of malaria in Sri Lanka in 1935 as a result of drought and intense breeding in the river bed pools are well known.

In Burma, *A. culicifacies* has similar breeding habits as in India (Khin-Maung-Kyi 1971). Throughout its range of distribution in Burma, the species is most abundant during the earlier part of the monsoon approximately between May and August. Later on heavy rains flush away the larvae but the conditions for breeding again become favourable after the monsoon.

Pal (1945a) made a study experimentally of the effect of physical and chemical conditions of water on oviposition, larval life, etc. again under laboratory conditions. Concentration of salinity upto 12 ppm did not affect the larvae of *A. culicifacies*. Higher concentrations were inimical.

In the Malnad area of Karnataka State, Brooke Worth (1935) made some observations on the ecology of the species and attributed the scarcity of *A. culicifacies* in the hilly high rainfall area of Hassan District to the paucity of suitable larval habitats such as quiet pools or slowly flowing streams of fresh water. It may be noted that *A. culicifacies* is comparatively a rare species in the entire region of the Western Ghats. The seasonal prevalence of *A. culicifacies* is characterized by low prevalence during southwest monsoon when a serious disturbance of the larvae occurs because of flooding.

Though, *A. culicifacies* is not generally associated with aquatic vegetation to any extent, Kachroo (1961) found in Bokaro reservoirs in the Damodar Valley in Bengal that when aquatic vegetation was controlled by herbicides and copper sulphate, there was much reduction in the breeding of *A. culicifacies* and *A. annularis*. This study suggested that *A. culicifacies* was breeding in the Bokaro reservoirs in the presence of aquatic vegetation.

Russell and Ramachandra Rao (1941b) made a study of the surface tensions of natural waters and found that no natural water had the low surface tensions required to drown larvae of *A. culicifacies*, though there were considerable variations.

Eggs

There is no record to show that eggs of *A. culicifacies* can survive in nature or in the laboratory in a desiccated condition like the eggs of certain Stegomyias. In this connection a study (unpublished) made by the present author would be of interest. It had been noticed that when the water was let into the main canals of the Cauvery-mettur Project in Pattukkottai in June many larvae, mainly first stage larvae; (some of the larvae could also have been those of *A. subpictus* or *A. vagus*) and a few of third and fourth stage larvae could be collected even one hour after the water flowed into the bone-dry canals. However, scrappings of the surface of the mud of canals prior to the flow did not yield any eggs nor were they expected to be found so easily, even if present. The presence of the first stage larvae in numbers in the fresh water posed a serious problem. Did the larvae flow down along with the

fresh water or did they hatch out from the eggs present on the soil? The latter was highly improbable because of severe summer temperatures prevalent in the months of May and June just prior to the flow of water. The soil was absolutely dry. In one year the author cycled up 22 miles on a major canal and found a few isolated pools of water persisting in the canal bed. They were well treated by larvicides so that any larvae in them were destroyed. In fact it could be stated with confidence that there was no breeding in the canal bed. Inspite of this, first stage as well as third and fourth stage larvae were flowing down with the current. There were also several places where the gushing water fell 4-5 feet down in cascades and still larvae were unaffected. Either there were eggs on the surface of the dry mud or the larvae flowed down with the current. The probability was that as the water was flowing down eggs were freshly deposited by the females and the larvae which hatched flowed down the current. A satisfactory explanation was not obtained.

The viability of eggs of anophelines when submerged in water was experimentally studied by Bhatia and Wattal (1958), using the eggs of *A. culicifacies, A. subpictus, A. annularis* and *A. stephensi.* The viability periods ranged from 0 to 96 hours, but the hatching ability decreased with time of submergence.

Sweet and Rao (1938) found no difference in egg measurements in *A. culicifacies* from five different places, viz. Mysore, Pune, Rajkot, Quetta and Larkhana (Pakistan). Similarly, Pal (1945) carried out a morphological study on eggs, larvae and pupae, from several places viz. Singhbhum hill and Korea State from east central India, Jabalpur in central India, Pattukkottai in Tamil Nadu, Lucknow and Delhi in the Gangetic plain and Sukkur in Sind. There were no morphological differences indicating the existence of two or more races. Comparative studies on Pattukkottai and Thanjavur delta were also made by the present author and no differences found.

Pupae

As in the case of larvae, pupae can withstand moderate temperatures but at 42.5°C pupae sank to the bottom and 43.3°C was the highest temperature which pupae could withstand (Pal, 1945). Pal also found that under laboratory conditions at 28° to 32°C, quite a substantial proportion of the pupae hatched into adults, but at a temperature of 36°C to 40°C only 30 to 40 per cent of the pupae hatched out.

Relation to Disease

A. culicifacies is perhaps the most dissected species of *Anopheles* in India for the purpose of determining natural malaria infections. Among the earliest recorded dissections were those by Cornwall (1902) (quoted from Bhatia and Krishnan, 1961) in Ennore, north of Madras, in which he found at 16.0 per cent sporozoite rate, among 25 females dissected. Stephens and Christophers (1902) (quoted from Bhatia and Krishnan, 1961) in the report of Malaria Commission of the Royal Society recorded a 4.0 per cent sporozoite rate among 252 dissected in Mian Mir near Lahore and a 8.6 per cent sporozoite rate among 69 dissected in Ennore, north of

Madras City. Since then dissections have been reported by a large number of workers in many parts of India and such high rates have not been recorded. Exhaustive lists have been given by Covell (1927), Bhatia and Krishnan (1961) and Horsfall (1972). There is no need to reproduce them here in toto but a few important comments are made taking into consideration only records in which large numbers have been dissected.

Sporozoite rates in naturally infected specimens have varied from about 0.1 as in moderately endemic areas in Pattukkottai where over 13,000 dissections were made (Russell and Ramachandra Rao, 1940b) to nearly 12 per cent in Sind (Pakistan); Covell and Baily (1932) found 11.8 per cent sporozoite rate out of 649 dissected in Sind (Pakistan) during an epidemic year (Bhatia and Krishnan, 1961). Corresponding oocyst rates have also been determined but quite often oocysts have not been looked for. In the list given by Bhatia and Krishnan the highest among the oocyst rates found were 16.6 per cent by Sinton (1925) in the Punjab, and 19.7 and 19.2 per cent found by Covell and Baily (1930) in Sind. The oocyst rates have been generally higher than sporozoite rates in most cases.

Zone-wise the following observations regarding infection rates can be made:

1. North Western Zone of India and also neighbouring parts of Pakistan and Afghanistan: moderate to high rates.
2. Western Ghats and Western coastal area: very low infection rates.
3. Deccan plateau: low to moderate rates.
4. Himalayan region: Very low rates.
5. East central India: Low rates.
6. Gangetic plains and Bengal deltas: Low rates.
7. Coastal Orissa, Andhra Pradesh, Tamil Nadu: Low to moderate infection rates.
8. Assam and neighbouring areas: Very low infection rates.

Russell and Ramachandra Rao (1940) analysed the dissection data for all parts of India gathered by many workers and summarized them (Table 31).

Table 31. Dissection data grouped by geographical regions prior to 1940

Area	*Glands dissected*	*Glands positive*	*Sporozoite index, per cent*
GROUP I			
Bengal and Darjeeling Terai	3,653	0	0
Singhbhum and Jeypore hills	8,801	0	0
Total	**12,454**	**0**	**0**

GROUP II			
Madras	14,129	9	0.063
Mysore	6,826	15	0.220
Travancore	4,079	18	0.441
Total	**25,034**	**42**	**0.168**
GROUP III			
Punjab, U.P. and Delhi	11,864	36	0.303
Total	**11,864**	**36**	**0.303**
GROUP IV			
Sind only	12,570	261	2.076
Total	**12,570**	**261**	**2.076**

As guts were not dissected in many cases, oocyst rates are not given.

A thorough and complete list of dissections of *A. culicifacies* traceable till then was also made by Senior White (1940c). He recorded the results of about 73,000 dissections made and tabulated them according to twelve subregions which he postulated in India. Generally speaking the observations were as follows:

Trans-Indus and Sri Lanka area.	—	Very active as a vector.
Between Indus and upper course of the Ganges, peninsular India south of the Godavari and Burma.	—	Sporozoite rate in first decimal place but it is a primary vector when it occurs in large numbers.
North Gangetic plain and in Satpura ranges.	—	Sporozoite rate in the decimal place, a weak vector.
In Chota Nagpur ranges, Jeypore hills, eastern coastal plain north of the Godavari.	—	Not found infected.
Bengal, Assam, N.E. region	—	Not of significance.

The observed facts supported the assumption of the existence of two morphologically indistinguishable races, one a vector and the other not so.

It is, therefore, seen that the highest rates of natural infections have been found in the north west zone of India and Pakistan. In the rest of the country, *A. culicifacies* is comparatively a weak vector, undoubtedly owing to its zoophilic habits. It makes up for this deficiency by occurring in very high numbers. Particularly in epidemic situations, even in the zones where it normally has a poor infection rate, higher infections have occasionally been found: for instance, the sporozoite rates

found by K.R.Rao in Hospet (Karnataka) 1.7 per cent; King and Krishnan in 1929 in Udayagiri (Andhra Pradesh) 2.4 per cent; Sweet and Rao (1931) Mysore State 10 per cent; Nursing *et al.* (1934) Mandya 1.0 per cent; Viswanathan (1936) Madakasira (Andhra Pradesh) 2.8 per cent; Deburca (1946) Jabalpur (Madhya Pradesh) 2.4 per cent; and Raghavan and Krishnan (1949) in Sriharikota (Andhra Pradesh) 1.8 per cent.

In the neighbouring countries, a sporozoite rate of 0.4 per cent in *A. culicifacies* out of 768 dissected was found in the eastern province of Afghanistan (Ramachandra Rao, 1951). The species has also been found infected in Iran and Muscat. Near Karachi, Quraishy *et al.* (1961) found a sporozoite rate of 14.8 per cent and an oocyst rate of 4.9 per cent in a study made in 1954. It is a vector of low efficiency in Nepal.

Further east in Burma, Khin-Maung-Kyi (1971) has recorded results of several dissections. Among the positive ones are those by Feegrade (1930*a* and 1930*b*) in Minbu District with a total infection rate between 1.5 and 2.4 per cent; Feegrade (1926*a*) in northern Shan State 0.9 per cent total infection rate; Tin (1928) in southern Shan State the total infection rate of 2.0 per cent; Singh (1940) at Kentung total infection rate of 4.1 per cent; Robertson (1941) in western Yunnan 3.8 per cent, etc. (All references quoted by Khin-Maung-Kyi, 1971). In more recent dissections carried out by Khin-Maung-Kyi and associates no infections have been recorded in several parts of Burma. Obviously *A. culicifacies* is a vector of some significance in Burma, but because of its generally low densities it is regarded as a secondary vector.

In Sri Lanka, the role of *A. culicifacies* in malaria transmission is very well known. It is perhaps the sole malaria vector in the island. It has caused very severe epidemics in the island most notable of which was in 1935. References may be made to Rajendram and Jayawickreme (1951) for further details. The sporozoite rates have ranged between 1.28 to 6.12 per cent.

When more than one vector is active in the same locality the relative importance of *A. culicifacies* has been studied by a few authors. For instance Issaris *et al.* (1953) working in the Terai area of Uttar Pradesh found both *A. fluviatilis* and *A. culicifacies* as vectors, but the latter was more important. *A. fluviatilis* was predominant during the pre-monsoon and post-monsoon periods whereas *A. culicifacies* was important in the monsoon periods.

In the Deccan plateau three species have been active in malaria transmission, viz., *A. culicifacies, A. stephensi* and *A. fluviatilis.* All have been found infected in nature and they seem to play similar roles. Their relative importance is determined only by their numbers (Viswanathan, 1950).

In Karachi City, Pakistan, Quraishy *et al.* (1961) found *A. subpictus* and *A. stephensi* in non-malarious areas whereas in three colonies where malariometric indices were high, *A. culicifacies* was found in large numbers with sporozoite rate of 14.8 per cent and oocyst rate of 4.9 per cent. While Karachi City was itself practically free from malaria, localities outside were highly malarious. This study was made in 1954.

There have been several epidemics of malaria in more recent years in parts of Gujarat State as for instance in the Rudramatha dam area in 1959 studied by the present author (unpublished), in the Kodiar Dam site in Amreli District (Bhatt *et al.*, 1962) and in the Gir forest area by Patel *et al.* (1961). In all these cases *A. culicifacies* was determined to be the vector. An extremely severe epidemic occurred in the Tharad and Vav Tahsils in North Gujarat in 1958 due to heavy rainfall. The epidemic was similar to that reported in Sind by Covell and Baily. No dissections were made but only *A. culicifacies* were found in enormous numbers. (Present author's unpublished date).

There has been some controversy regarding the role which *A. culicifacies* plays in malaria transmission in eastern and north-eastern part of the Indian peninsula. Extensive dissections made by Senior White and colleagues and also by workers, like Iyengar in Bengal and Anderson and Viswanathan in Assam, have shown that no infections or low rates were found in this area, not dissimilar to what was found in the irrigated areas in Tamil Nadu State. Subba Rao and Appa Rao (1945) concluded that this species played a definite, though secondary, role in transmission of malaria in the east central and eastern regions.The role of *A. culicifacies* in these parts of India has been overshadowed by the role of such vectors as *A. fluviatilis, A. balabacensis, A. minimus, A. sundaicus, A. philippinensis* and *A. annularis.* In the Deccan as already stated it shares the transmission with *A. stephensi mysorensis* and *A. fluviatilis.* In the western and north western part of the country, however, *A. culicifacies* is the sole vector in most of rural areas but in urban centres it is replaced by or associated with *A. stephensi.*

Many studies have been made on successful experimental infections of *A. culicifacies* with malaria plasmodia. A notable one was by Siddons (1944). He stated that the upper limiting value of temperatures for the development of Indian strains of *P. vivax* and *P. falciparum* in *A. culicifacies* lay between 90° and 94°F; the optimum temperature range was between 70° and 86°F. He found that *A. culicifacies* also could be an efficient vector of *P. malariae,* the extrinsic incubation period in *A. culicifacies* being 14 days at 80°F to 22 days at 70°F. The intrinsic incubation period in man was 30 days. As is well known *P. malariae* can persist in the human body for very long time.

The role of *A. culicifacies* in transmission of non-human malaria, particularly monkey malaria, has not been established. Jaswant Singh *et al.* (1949) found no infections when *A. culicifacies* females were fed on *P. knowlesi* in monkeys.

There is no evidence of *A. culicifacies* playing any role in human or animal filariasis.

In Nasik District of Maharashtra, Viswanathan and Bhatt (1948) found a species of *Trypanssome* (*T. kalwanensis*) in the salivary glands of a female *A. culicifacies.*

Control

The control of *A. culicifacies* specifically is not dealt with here as in the case of other major vectors such as *A. fluviatilis, A. minimus, A. balabacensis, A. sundaicus* and *A. stephensi.* The history of malaria control in India is largely a history of

the control of *A. culicifacies* till the commencement of the DDT era. Being a species which is ubiquitous in its breeding habits there were no special control measures for it. The details are referred to in the section on "Landmarks in the control of anophelines". However, it is worth mentioning that pyrethrum space-spraying seemed to be most efficient when directed against *A. culicifacies*. DDT indoor residual spraying has been effective throughout India, but *A. culicifacies* has now become resistant to DDT, dieldrin and HCH in many parts of the country. It is also resistant to malathion in a few districts of Gujarat and Maharashtra States. For details, see chapter on "Insecticide resistance".

Anopheles dthali Patton, 1905

Type locality: Aden.

Type: Unknown, but the type of a synonym *wardi* Leeson and Theodor, 1948, is stated to be in the British Museum.

Taxonomy/Distinguishing characters: Close of *A. culicifacies*, but easily distinguished by the wing. Except for costa, sub-costa and vein 1, it is completely dark. It has a shining mesothorax.

Distribution: Very widely distributed in West Asia (Pakistan and to the west); northern Mediterranean region as far as Morocco, Ethiopia, etc. In India there is only one record in Kashmir.

Bionomics/Ecology: The adults have been easily collected in houses, stables, tents, etc. It is known to bite man. Larvae are found in wells, river bed pools, springs, etc. Margins of rivers and streams covered by pebbles, rice fields, seepages, etc. seem to be its preferred breeding places. Nothing is known about the bionomics of *A. dthali* in India because of its being a rare species. But in the southern Iran, a recent study by Manoucheri and Rohan (1975) has added much information.

The anthropophilic index ranges from 1 to 25 per cent. Its peak man-biting period is between 20.00 and 21.00 hours.

Relation to disease: *A. dthali* has been incriminated as a vector on epidemiological grounds. The recent work of Manoucheri and Rohan (*loc. cit.*) indicates that it is a seasonal vector. The sporozoite rates found in three different areas were 2/96, 1/100 and 3/39. According to Indian standards, these are quite high rates, but the Iranian authors recorded them as low infection rates.

A. dthali was detected to be a new secondary vector of malaria in southern Iran (Manoucheri *et al.*, 1972).

Anopheles jeyporiensis James, 1902 and variety *candidiensis* Koidzumi, 1924

Type locality: Patingi, Jeypore Hill Tracts, now in Orissa State, India.

Type: British Museum.

Type locality, Variety: Lake Candidus, Taiwan.

Type, Variety: Unknown.

Taxonomy: A synonym of the type form named *jeyporensis* was described by Theobald in 1903 from the same locality, viz. Jeypore Hill Tracts.

A synonym of the variety is *tonkinensis* of Toumanoff 1931 with the type locality in Indonesia.

Much confusion and misidentification has occurred in the past in the identification of the type form and the variety. Further many identifications of var. *candidiensis* may be of *A. moghulensis.*

Distinguishing characters: Type form: Adult, a medium sized mosquito. Mesonotum with many white scales; tarsi with narrow but distinct pale bands. From *A. moghulensis* which it resembles it is distinguished by the absence of a line of overlapping broad white scales on side of thorax in front of the wing root.

Larva: The outer clypeals with a large number of pinnate branches; one long pleural hair on metathorax simple. Palmate hairs well developed on metathorax and abdominal segments I to VII.

Variety: Var. *candidiensis* may be distinguished from the type form by:—

Adult: The apical pale band on palpi longer with the intervening dark area between the apical and preapical pale band narrower. The preapical pale band is also longer than in the type form. The preapical dark spot on vein 1 more frequently interrupted. Fore and mid tarsi with banding on segment 3 more frequent than in type form.

Larva: Inner clypeal hair with fewer and thicker branches than in the type form.

Distribution: TYPE FORM: India, Nepal, Bangladesh, Burma and 'Indochina'. Also references to occurrence in China. Reid (1968) says that it occurs in Thailand but not in Thai peninsula. The variety has a very similar distribution but more common in the eastern regions than in the western regions.

In India, the type form is widely distributed except in the extreme north and north west, viz. Kashmir, Punjab, Haryana and Himachal Pradesh. Though reported from Gujarat and Rajasthan, the numbers are scanty.

The varietal form was, (till Christophers, 1933), not regarded to occur in the western and southern zones of India. However, several more recent studies have shown small numbers in the hilly regions of south India and Western Ghats.

Prevalence

The species varies considerably in its prevalence. It is the most common anopheline in the Western Ghats of Karnataka and Kerala. In fact in North Kanara it formed nearly 40 per cent of all adults collected. High densities have also been found in Hassan District of Karnataka and Wynaad District in Kerala. It occurs in abundance in most of its range, but not always so predominantly.

It seems to be a species which occurs in all months of the year, but as it is liable to be flushed away by monsoon rains, it is more prevalent in the post monsoon months.

Adult Bionomics

Being a species not of much significance as a vector, it has received comparatively

little attention as regards its bionomics.

Daytime resting habits: The species is found in large numbers in cattle sheds and to a lesser extent inside houses. This has been recorded by almost all workers in India. However, in Burma the species has never been taken indoors though collected during night catches (Khin-Maung-Kyi).

Much of the confusion and conflicting reports on bionomics may be due to the failure to distinguish the type form from the variety.

Senior White (1946) has found a few specimens of the type-form resting on the muddy banks of streams in east-central India. Senior White *et al.* (1945) also noted that the type-form stayed inside premises only during the earlier part of the gonotrophic cycle.

The adults of the variety seem to behave similarly.

Feeding and biting habits: In Karnataka, Nursing *et al.* (1934) found the species to enter huts mainly between 04.00 to 06.00 hours quite unlike the observations in Burma (Macan, 1950) where it entered the huts between 22.00 and 24.00 hours and from between 01.30 and 03.30 hours and then left the premises. In N. Kanara also it is an early biter.

That the species bites man is well known but the proportion biting man and cattle has varied considerably. However, it has much higher anthropophilic indices than some proved vectors like *A. culicifacies*. In Burma, Khin-Maung-Kyi (1971) records that when cattle are not present many bite man. The species seems to exhibit no special attraction to cattle or man, biting them equally if the opportunity arises.

Little information exists on other aspects of adult behaviour.

Larval Ecology

Grassy edges of slow moving streams and channels as well as grassy edges of shallow tanks are ideal breeding places. Senior White and colleagues have found the species in seepage fields; rice fields are attractive when fallow but become rapidly unfavourable as the plants grow up. In North Kanara the present author has found the species in a variety of breeding places with clean water, with a preference for channels with marginal vegetation.

There appears to be no difference in breeding habits between the type-form and the variety.

Relation to Disease

In several series of dissections in India a few infected specimens have been found in the following places:—

Karnataka, Jeypore Hills (Orissa), Kerala and Assam. The Jeypore Hill Tracts have provided most of the infected specimens following the work of Senior White and colleagues. Infection rates have ranged from 0.04 per cent in Assam to 3.0 per cent in Jeypore Hills. It is not clear whether the infections were in the type-form or

variety *candidiensis*, probably they were in the latter which is more abundant in the eastern parts of India than in the west and south.

However, in other countries particularly China, Burma and 'Indochina' many more infected specimens have been found. In China infection rates upto 5.4 per cent have been recorded in the Yunnan Province and Hong Kong.

In Burma infection rates upto 5.5 per cent have been found in the Burma-China border. It is, however, regarded a vector secondary to *A. minimus* and perhaps to *A. balabacensis*. Strangely Harinasuta *et al.* (1976) do not list *A. jeyporiensis* as a vector in Burma.

Most of these infections particularly in China and 'Indochina' are undoubtedly in the variety *candidiensis*.

In India also, the variety is a better vector than the type-form.

Good reviews of the bionomics and relation to disease are by Wattal (1961) and Khin-Maung-Kyi (1971).

Anopheles majidi McCombie Young and Majid, 1928

Type locality: Coorg, Karnataka State, India.

Type: National Institute of Communicable Diseases Delhi.

Taxonomy: This species was first described as a variety of *A. karwari*, but Puri (1928) determined it as a distinct species.

Distribution: India, Nepal.

In India, it has a most discontinuous distribution; Bengal (Darjeeling and Jalapaiguri Districts), Karnataka (Coorg, Kadur and Hassan District), Kerala (Wynaad District), and Tamil Nadu (Nilgiris District). In none of the extensive collections made in North Kanara District by Bombay State workers and by Jaswant Singh and Jacob, were any specimen found. It has not been collected anywhere in the Western Ghats of Maharashtra.

Distinguishing characters: A small to medium sized mosquito similar to *A. karwari*, but differs from it by only two white broad apical bands and a narrow basal band on palpi while in *A. karwari* there are four pale bands, three apical and one basal. From other related anophelines it is distinguished by (*a*) unspeckled legs, and (*b*) hind tarsi with only one segment completely white.

Adult Bionomics

Little information is available. Adults have been collected in houses and cattle sheds in Wynaad and Darjeeling area.

Larval Ecology

The species was found breeding in "grassy slow running streams" by McCombie Young and Majid (1928). Iyengar (1929) has recorded the species from open drains in tea gardens and fallow rice fields.

In Sakleshpur area of the Malnad in Karnataka, the species is very rare and has been taken as larvae only between January and March (Brooke Worth, 1953).

Relation to Disease

A very rare species and unlikely to be involved in transmission of human malaria. A few dissections made in Karnataka, Wynaad, Nilgiris and Darjeeling, have been negative.

Anopheles subpictus Grassi, 1899 and var. *vadakadiensis* Doraisamy, 1963

Type locality: Calcutta.

Type: Rome University Museum.

Taxonomy: For a long time species was known in India as *A. rossi* Giles, 1899 till it was named as a synonym by Edwards (1920). Another synonym is *A. error* Theobald, 1903. Both *rossi* and *error* had their type localities in India. There was much confusion till King (1932) clarified the situation (see also Christophers, 1933). Stone *et al.* (1959) had listed two varieties namely, *indefinitus* (Ludlow) and *malayensis* Hacker the former with a wide distribution in the Philippines, Indonesia, China, Taiwan, and Mariana Islands and the latter restricted to Malaysia and Sulawesi. But in the latest edition of the 'International Catalog' by Knight and Stone (1977), *indefinitus* has been raised to the status of a species, following Reid (1966) and *malayensis* is designated as a synonym of *indefinitus*. *A. indefinitus* has not been reported from India.

A new variety of *A. subpictus* has been added; viz. var. *vadakadiensis* Doraisamy, 1963, from India.

Type locality: Vadakadu, Rameswaram Island, Tamil Nadu. The type was stated to be deposited in National Malaria Institute (Sic).

Distinguishing characters: Adult is a medium sized mosquito with the general pale colouration characteristic of the members of *Pseudomyzomyia*. The tarsi of fore legs have broad pale bands; femora and tibia are not speckled and the palpi of female has the dark pre-apical area about equal to the apical band. It is distinguished from the closely related *A. vagus* by the palpi. In the latter, the apical pale band is quite long and the pre-apical dark area and the preapical pale band are very narrow. Both are distinguished from *A. sundaicus* by having no speckling on the legs. The adult males of *A. subpictus* and *A. vagus* cannot be distinguished.

Larva: The outer clypeals and posterior clypeal hairs a little longer than half the length of inner clypeal hairs, differing from *A. vagus* in which the outer and posterior clypeal hairs are very short, less than 1/3 of the inner clypeal hairs. Palmate hair is not differentiated on thorax and filaments of the abdominal palmate hair are short; both long pleural hair on mesothorax simple. Variety *vadakadiensis* has the inner clypeal hairs distinctly bifid.

Distribution: *A. subpictus* is very widely distributed in the Oriental region

extending from Afghanistan, Iran in the West to New Guinea, Mariana Islands in the East. It occurs in Sri Lanka in the South and China in the North. In India *A. subpictus* occurs in all the mainland zones. It is found in the Lakshadweep Island, but not in the Andamans.

Prevalence

Perhaps *A. subpictus* is the most abundant species in the anopheline fauna in large parts of peninsular India and north-west zone. It is found at all altitudes, excepting the highest. Though its numbers are small, it occurs in many parts of the Himalayas. In the recent survey of the Himalayas, Ramachandra Rao *et al.* (1973) have recorded it from several hill districts of Uttar Pradesh at heights ranging upto 1910 m, in Nainital, Pithorgarh and Chamoli. It was not collected in the hill districts of Himachal Pradesh. However, Jacob (1950) has recorded it in certain parts of Kashmir. Varma and Mahadevan (1970) had not found any specimen in the Sikkim and West Bengal areas of the Himalayas. In the Nilgiris in the South, it has been found at an altitude of about 1,800 m at Coonoor (Puri, 1946). In the entire region of the Western Ghats, it yields the dominant position to *A. jeyporiensis* (Jaswant Singh and Jacob (1944); Covell and Harbhagwan (1938); Viswanathan (1950) and Brooke Worth (1953). In peninsular India, it occurs in close association with *A. culicifacies* and *A. vagus.*

A. subpictus gradually declines in abundance proceeding eastwards in India. At one time it was thought that it did not prevail east of Burma (Christophers, 1933), but now it is known that it occurs as far east as New Guinea overlapping in distribution *A. indefinitus* in the eastern regions. In Burma, it has been reported from many localities. Neither Rajagopal (1976) in Burnihat (Assam-Meghalaya) nor Sen *et al.* (1973) in Tirap region of Arunachal Pradesh found any specimen of *A. subpictus* in their night collection.

In the studies made in 1978 on long term changes of the *Anopheles* species prevalent in Pattukkottai area, Rajagopalan *et al.* (1979) have found that neither DDT operations (of course now long suspended) nor any other measures had reduced the prevalence of *A. subpictus.* In fact relatively speaking *A. subpictus* had increased several fold. While in 1940s its numbers was about the same as or only slightly higher that of *A. culicifacies,* it appears that in 1978 it was 18 times more prevalent. It would be interesting to find out the cause for such a big change.

The species occurs in all seasons of the year depending upon the availability of breeding places. In South India, Russell and Ramachandra Rao (1941) found that *A. subpictus* was predominant during the irrigation season and adult densities of over 50 per man hour were found in human and animal dwellings in the months of August to November. Similar was the prevalence of *A. culicifacies.* There is an unconformed report of hibernation of the species (Choudhury, 1931). In recent studies by Aslamkhan, Reisen and others in Pakistan Punjab, the species was practically absent in dwellings during the cold season.

Adult Bionomics

Day-time resting habits: *A. subpictus* adults are very commonly found in houses and cattlesheds. Often they have also been collected outdoors. Though numerous collections have been made, the biology of this species has not received much attention because of its having no or little role in malaria transmission. The species has no reluctance to enter magoon type of traps because 17,181 females were collected in such a trap during 350 night collections, the largest number of any species collected.

Host preferences: It feeds predominantly on cattle and other domestic animals (Russell and Jacob, 1939, Afridi *et al.*, 1939 etc.). In their extensive studies on the host preferences of Indian anophelines, Bruce-Chwatt *et al.* (1966) have pointed that out of 2,607 precipitin tests positive,only 241 (9.2 per cent) had human blood. Even this is much higher than that usually found in South India. In certain circumstances in the absence of cattle in a locality *A. subpictus* may bite man in larger numbers. Even in very early studies precipitin tests had shown that it does bite man. Roy (1943) has stated that in the Salt lake area of Calcutta (now completely swallowed up by urban Calcutta) the species had an anthropophilic index of 25 per cent based on precipitin tests.

There are very few comparative biting studies made simultaneously on man and cattle for drawing meaningful inferences regarding the relative attraction of man and cattle. In a study made by Aslamkhan and Salman (1967) in Pakistan, they found that using a man and a cow as baits, between 18.00 and 21.00 hours from April to October 1964, in the Changa Manga National Forest, near Lahore, only 36 females of *A. subpictus* were collected on man while 1,842 were collected biting the cow, the ratio of man to cow being 1:51. These figures obtained during a total of 336 man-hour collection perhaps, indicate the host preference of *A. subpictus* most clearly.

Nocturnal habits: Reisen *et al.* (1976) have found generally smaller numbers of *A. subpictus* in cattlesheds during nights than during day-time near Lahore. But larger numbers were found between 18.00 and 19.15 hours and 05.00 to 07.15 hours than at other times. Their figures of collection of mean numbers of *A. subpictus* (females and males) collected resting in cattle sheds were as follows:—

Time	*Females*	*Males*
07.00 hours	19.5	3.5
12.00 hours	8.5	1.5
18.00 hours	11.0	3.5
18.00 to 19.15 hours	8.5	0.6
20.00 to 22.15 hours	0.9	0.1
23.00 to 01.15 hours	0.9	0.1
02.00 to 04.15 hours	1.2	0.2
05.00 to 07.15 hours	7.6	5.8

These are very similar to the patterns of resting during various hours of day and night of *A. culicifacies* also in which the collections made during daytime ranged

from 32.00 females and 6.0 males to 57.5 females and 2.0 males while night collections were generally 1.7 females and 0.8 males or less.

Apparently, the outdoor resting by the species is not of the same order as with other species including *A. culicifacies.* This may be one of the reasons why large numbers of this species are collected in indoor resting places. But there are no reliable data.

Studying the biting rhythm of *A. subpictus* in Lahore, Pakistan, Reisen and Aslamkhan (1978) have pointed out that *A. subpictus* mostly fed prior to midnight with an occasional secondary pre-dawn peak. Reuben (1971) working in Vellore reported that most biting occurred btween 21.00 and 22.00 hours. In a very recent study by Rajagopalan *et al.* (1979) in Pattukkottai area the number biting cattle at different times of night were as follows:—

18.00 — 20.00 hours . . .	68
20.00 — 22.00 hours . . .	123
22.00 — 24.00 hours . . .	52
00.00 — 02.00 hours . . .	114
02.00 — 04.00 hours . . .	154
04.00 — 06.00 hours . . .	104

The number of nights of collections were small, but it will be seen that the adults were found biting throughout the night with a slight preponderance after midnight. They also found very similar trends in *A. nigerrimus* which is known to have a strong preference to bite during crepuscular periods. Roy (1940) had also found *A. subpictus* females feeding at all times at night while *A. annularis* fed mainly in the first part. The studies need to be repeated.

Flight and dispersal: *A. subpictus* is generally regarded as a strong flier, better than *A. culicifacies,* but reliable information does not exist in India. In Indonesia, it has been found flying upto 1.5 kms by many workers. But it may be noted that in that country *A. subpictus* and *A.indefinitus* have been often confused with.

Swarming and mating: The swarming and mating of *A. subpictus* has been described by Venkat Rao *et al.* (1942) in Orissa in the Chilka Lake area. The swarms consisted of as many as 5,000 specimens comprising both *A. subpictus* and *A. sundaicus.* The swarms were about 3 m long by 1 m wide and 0.3 m high. In the Philippines swarms of several thousands have been seen (Bank 1919 quoted by Horsfall, 1972) but they could be of *A. indefinitus* which till recently was considered as a variety of *A. subpictus.*

Roy's (1940) observations in Calcutta showed that *A. subpictus* had not readily mated in cages of 2ft×2ft×2ft×1ft (60×60×30 cms), while *A. stephensi* and *A. annularis* readily did so. Perhaps the mating behaviour of *A. subpictus* is somewhat different even in nature from that of *A. annularis.* Roy (1940) found that nearly 99 per cent of wild caught *A. annularis* were found to have already mated while large proportions of similarly caught *A. subpictus* were unmated though they had taken blood meals.

Gonotrophic conditions: The periodicity of feeding is not precisely known, but it

seems to conform to that related species like *A. sundaicus, A. vagus,* etc. and it feeds normally once in 48 hours. The earlier work of Christophers on egg development made on *A. subpictus* had shown that the first batch of eggs required about 6 days.

Longevity: Mehta (1934) working in Karnal, under laboratory conditions Haryana, concluded that at 40° C females of *A. subpictus* did not survive more than 24—50 hours when the R.H. ranged from 50 to 90 per cent. But the longevity increased when the temperature was lowered to 35° C. At 30° C and 70 per cent R.H. females lived 6—14 days and at 25° C upto 20 days. High humidity above 90 per cent was injurious. In nature, he thought *A. subpictus* lived from 5—11 days at 30° C.

Larval Ecology

Breeding places: *A. subpictus* is perhaps the most ubiquitous of all species regarding its breeding places. It occurs practically in every type of breeding place except the most polluted or contaminated. However, there must be a few types which are particularly favourable to it because in the hills and foothills the species is somewhat less abundant though present. For example, in the Western Ghats, it occurs in far lesser numbers perhaps due to the absence of the preferred breeding places.

It occurs in flowing or stagnant water, clear or turbid water, water with or without vegetation, unshaded or slightly shaded places, wells, borrowpits, channels, lake margins, ponds, tanks, ground pools, fallow and freshly flooded rice fields (becomes less prevalent as the rice plants grow up), even in cement cisterns, fresh or brackish water, etc. As an example of this catholicity, the findings in Pattukkottai by Russell and Ramachandra Rao (1940b) may be cited. They recorded 35,362 *A. subpictus* larvae collected in one year (1937-38) (Table 32).

Table 32. The breeding places and the abundance of *A. subpictus* in Pattukkottai

	No. of breeding places searched	*Number positive for sub-pictus*	*Number of larvae*	*Percentages*	
				Breeding places	*Larvae*
Wells—all types	1,240	375	7,167	30.2	18.8
Tanks	1,523	729	10,168	47.9	46.7
Rice fields growing	779	283	3,801	29.9	23.0
Waste irrigation water	668	350	4,975	52.2	32.2
Irrigation channels	769	200	1,739	26.1	13.1
Borrowpits	396	249	4,348	62.9	41.5
Field channels	507	170	1,318	33.5	16.9
Rice field fallow	336	218	3,569	64.9	50.6
Ditch	299	153	2,013	51.2	31.4
Seepages	173	93	2,035	53.8	39.1
Rain water pools	124	82	1,893	66.1	55.5
Hoof marks, cart tracks	75	48	518	64.0	29.3
Total	6,033	2,453	38,362	40.7	27.7

Obviously, the species is very widely distributed in many types of breeding places. But fallow rice fields, borrow pits, rain water pools, all generally with turbid water, were the most frequented places; irrigation channels were the least frequented, though about a quarter of channels had the larvae. These findings are very similar to the observations made in most parts of India where the species is prevalent.

The above figures may be compared with those relating to *A. culicifacies* to show the subtle differences in habits between the two species so closely associated in nature.

A. subpictus can breed well even in saline waters as observed by James (1902) and Chalam (1924). Chalam found the species in water with salinity equivalent to 90 per cent of sea water. Covell and Pritam Singh (1942) have shown that *A. subpictus* can tolerate quite high degrees of salinity along with *A. sundaicus*. In the Salt Lake area of Calcutta it had been found breeding along with *A. sundaicus* (Sen, 1938). The present author had collected *A. subpictus* larvae in the coastal swamps in Adiramapatnam near Pattukkottai between 1936-1942. In Java also the two species have been associated with brackish water.

The larvae of other species with which it has been most associated in breeding places in South India were *A. culicifacies* and *A. vagus*. The next in order were *A. pallidus* and *A. annularis*. There was no species with which it was not associated except, *A. tessellatus* which, however, was uncommon. These studies which were in considerable detail generally confirmed most of the previous findings of the breeding places recorded or referred to by Christophers (1933).

Vegetation: Sen (1941 and 1948) made many observations on anopheline larvae in relation to vegetation. He found fallow rice fields to be the most prolific sources of *A. subpictus,* but the prevalence declined when the plants reached about two feet in height (0.6 m). Regarding vegetation he found that waters with *Spirogyra* and *Pistia* and water hyacinth were preferred to those with abundant growths of *Utricularis, Hydrilla* or *Najas*. The growth of the aquatic plant *Chara,* often regarded as inimical to the breeding of anopheline larvae, was found by Neogy and Kachroo (1956), in the Damodar Valley area, not to inhibit anopheles breeding, particularly of *A. subpictus*. The species of *Chara* was *C. zeylonica.*

Slight morphological differences between forms growing in fresh and salt water have been described (Sundaresan and Appa Rao, 1943). The details are given under *A. sundaicus.*

The observations on *A. subpictus* in other countries formerly attributed to this species have become somewhat unreliable because of the confusion existing between *A. subpictus* and *A. indefinitus.* Reid (1968) has pointed out when dealing with the habits of *A. indefinitus* that the larvae of the species "are typically found in grassy pools, ponds and ditches and not in the small muddy pools favoured by *A. subpictus* in India; in Malaya the latter sites are occupied by *vagus*". In Malaysia and Java, *A. subpictus* is confined to the coastal regions, the interior places being occupied by *A. vagus.*

Relation to Disease

A. subpictus is not considered to be a malaria vector of any consequence in India. Roy reviewing the previous observations has mentioned findings of Strickland, Chowdhury and Chaudhury, 1933, of two infections out of 10,452 dissected in Darjeeling Terai. Subsequently, Russell and co-workers have found infections in South India as follows:

	Dissected			Positives		
	Guts	*Glands*	*Total*	*Guts*	*Glands*	*Total*
Ennore Russell, Ramachandra Rao and Jacob (1939)	3,853	4,722	4,728	2	0	2
Pattukkottai Russell & Ramachandra Rao (1940)	12,360	13,017	13,277	2	1	2
			Total	**4**	**1**	**4**

Recently, a high prevalence of malaria in a coastal village near Pondicherry due to *A. subpictus* has been reported. Panicker *et al.* (1981) detected 45 gut and 2 gland infections among 3,752 females of *A. subpictus* dissected between April and July, 1981. There were no other known vectors present and *A. subpictus* accounted for 98% of all anophelines collected in houses. The species was biting man and 14% of the stomach bloods were positive for human blood.

A. subpictus has been infected with malaria plasmodia in the laboratory. Roy (1943) making a comparative study of experimental infections in *A. subpictus* salt water and *A. stephensi* in Calcutta made the following observations. The infections were as follows:—

	No. dissected	No. positive	
		Gut	*Gland*
A. subpictus			
P. vivax	15	5	0
P. falciparum	50	16	5
P. malariae	5	0	0
Mixed—*vivax* and *falciparum*	14	7	2
A. stephensi			
P. vivax	18	13	10
P. falciparum	52	30	22
P. malariae	7	3	0
Mixed—*vivax* and *falciparum*	9	3	7

These studies while showing that *A. subpictus* could be infected in the laboratory, it had a much lower susceptibility to infection than *A. stephensi.* Based on these findings, Roy concluded that in certain parts of India, which are very humid,

infected specimens of *A. subpictus* in small numbers are likely to be encountered. But the danger of this species from the malaria transmission point of view was negligible.

Recent experiments by Dass *et al.* (1979) in Salem, Tamil Nadu, have shown that, when fed on the same series of 13 human volunteer donors with gametocytes, not a single one of 212 *A. subpictus* became infected, while out of 272 *A. stephensi*, 58 showed gut infections and 73 gland infections (total 93 infections). *A. stephensi* became infected with almost every donor. Both *vivax* and *falciparum* were involved and *A. stephensi* was infected by both.

Apart from its general refractoriness to infections, how much of the vectorial incapacity would be due to other causes like low longevity in nature is not known. There is nothing to indicate whether the species is long lived or not in nature. Roy (1943) found in the laboratory and average mortality of 27 per cent in 4 days, 84 per cent in 10 days and 97 per cent in 15 days. There can be no doubt that in nature the mortality rates would be considerably higher. Mehta (1934) felt that it lived from 5 to 11 days in nature. It may be recalled that with *A. culicifacies* in conditions simulating nature Russell and Ramachandra Rao have found that 50 per cent died in two days and only about 3 per cent survived 10 days.

Records of dissections in other countries show that infected specimens have been found in Indonesia. There is much speculation now whether the infections were in *A. indefinitus* or in *A. subpictus sensu stricto*. Infections as tabulated by Horsfall (1972) are all gut infections. Reid (1968) says "on the south coast of Java Sundararaman *et al.* (1976) found *A. subpictus* to be a minor vector of malaria." They found no infections in *A. indefinitus*.

It is probably only in Indonesia that *A. subpictus* and *A. indefinitus* have some role in malaria transmission. Even in the very informative and upto date article edited by Harinasuta *et al.* (1975) regarding the vector in Southeast Asia, *A. subpictus* has been mentioned as a vector (not as a major one) only in Indonesia.

In the Bola regions of Indonesian Timor recently Lien *et al.* (1975) found three out of 94 *A. subpictus* and one out of 19 *A. barbirostris* positive for sporozoites. But it is not clear whether it was *A. subpictus* s.s., or *A. indefinitus*. *A. indefinitus* was found in Guam in 1969 when it became established there. Though there was much malaria, Pratt and Siren (1971) thought that *A. indefinitus* was probably not a good vector.

So far as bird malaria is concerned, none of the 75 *A. subpictus* fed on sparrows infected with *P. relictum* became infected, while 6,566 out of 7,516 *Culex fatigans* and 20 out of 24 *C.(lutzia) fuscanus* developed sporozoites (Jaswant Singh *et al.*, 1951).

There is no epidemiological or experimental evidence to suggest that *A. subpictus* has any role in the transmission of monkey malaria.

A. subpictus has been found both infected in nature and experimentally infected by filaria parasites (*W. bancrofti*) (Raghavan, 1964).

Two arboviruses, namely *Arkonam* and *Batai*, have been isolated from *A.subpictus* in India; *Arkonam* in North Arcot District of Tamil Nadu and *Batai* from a

rural area near Pune in Maharashtra. (V.R.C. Annual Reports). Recently *A. subpictus* has been found infected with Japanese encephalitis virus in Karnataka (N.I.V. unpublished reports).

A. subpictus has been experimentally infected into West Nile, a group *B* arbovirus (VRC reports).

Control

There have been no special programmes to control *A. subpictus* but invariably when attack was made on other species such as *A. culicifacies, A. subpictus* has also been affected as they breed or take rest in similar situations. DDT was very effective in reducing density for some time, but soon lost its effectiveness. *A. subpictus* was perhaps the first *Anopheles* to become resistant to DDT in India. Sharma and Krishnamurthy (1958) first detected development of DDT resistance in *A. subpictus* in Delhi State in 1954. Macdonald and Nasir (1961) reported a definite resistance to DDT in *A. subpictus* in Lahore.

Anopheles vagus Donitz, 1902

Type locality: Fort De Kock, Sumatra, Indonesia.

Type: Not available according to Christophers, (1933) but Knight and Stone (1977) state that the specimens are in Zoological Museum of Humboldt University, Berlin, G.D.R. This has been confirmed.

Taxonomy: There has been much confusion in the taxonomy of this species closely related as it was to *A. subpictus* and its varieties. Even today the males of the two species cannot be distinguished.

There are two recognised subspecies: ssp. *limosus* King, 1932 (Philippines and Borneo). ssp. *albino* Stoker and Waktoedi, 1949 (Indonesia), neither of which occur in India.

Distribution: Extensive in the Oriental region, from western India (Gujarat) to New Guinea and Moluccas, Sri Lanka and Hong Kong.

In India: Throughout the country: does not extend to western countries and to Pakistan. Occurs in the Andamans.

Distinguishing characters: Very closely resembles *A. subpictus* but can be distinguished both in the adult (female only) and larval stages. In the adult female the tip of the palpi has a long apical pale band followed by a narrow pale band interrupted by a short dark area, while in *A. subpictus* the apical pale band is of normal width and the intervening dark area is as wide as the apical band.

In larvae, the outer and posterior clypeal hairs are very short hardly 1/4 to 1/3 length of the inner clypeal.

Prevalence

A fairly common species occurring in association with *A. subpictus* in the eastern and southern parts of India, it is not common in hills of the Western Ghats. While Russell and Ramachandra Rao (1941a) in Pattukkottai found 14,964 adult females of *A. vagus* as against 46,468 of *A. subpictus,* Brooke Worth found 408 adults in Hassan District as compared with 431 *A. subpictus.* In old Bombay State, in the

western parts of India, the number of *A. vagus* females collected in several years was only 6,561 as against 164,880 of *A. subpictus* (Viswanathan, 1950). In eastern India, *A. vagus* becomes more common than *A. subpictus*. For example in a survey by Paul *et al.* (1936) in Goalpara District of Assam, they dissected 218 *A. vagus* against only six *A. subpictus* indicating the comparative prevalence. In all-night collections made in Burnihat, Meghalaya, in 1968, Rajagopal (1976) found only 83 adults biting cattle against 2,130 adults of all species—a rather low prevalence.

In Burma, it is the commonest species of anophelines found in all types of topography, far more prevalent than *A. subpictus*.

Adult Bionomics

Resting habits: The adults are easily collected in houses and cattle sheds. In Pattukkottai they were found in about equal numbers in human dwellings, mixed dwellings and cattle sheds (6.1, 6.0 and 6.5 per man-hour respectively). Generally cattle sheds are regarded as more attractive because of zoophilic habits. In Burma, the adults found in houses are comparatively small in number (Khin-Maung-Kyi, 1971). No information exists on the degree of outdoor resting, but seems to occur only to a limited extent.

Biting habits: Very few studies have been made on actual time of biting, but in Burma it has been found biting about midnight.

Host preferences: *A. vagus* is regarded generally as a cattle-feeder, occasionally biting man. Reid (1965) summarizing the results of precipitin tests carried out by others have given the following figures:

Assam, Ramsay *et al.* 1936	. . . 1 per cent	with human blood
"Indochina", Toumanaff 1936	. . . 4 per cent	,,
Indonesia, Walch, 1932	. . . 1 per cent	,,
Malaysia, Hodgking 1956	. . . 3 per cent	,,
Malaysia, Whatton, 1953	. . . 7 per cent	,,

Reid (1968) placed *A. vagus* at the bottom of the list of the species in Southeast Asia regarding man-biting habits.

Ramsay *et al.* (1936) examined 593 stomach bloods out of which 470 were positive for cattle blood and only 4 for human blood.

Larval Ecology

A. vagus is a species commonly associated with *A. subpictus* in breeding places, having similar breeding preferences. However, *A. vagus* breeds more intensely in muddy waters. It breeds in borrowpits, muddy ponds, rain water pools, hoof marks, cart tracks, etc. In Pattukkottai area 4,024 larvae were collected in 703 breeding places out of 6,033 searched.

The breeding places in order of preference were:

	Per cent of times
Hoof marks, cart tracks	33.3
Rain water pools	30.7
Ditches	22.4
Rice fields—fallow	18.2

Borrowpits	17.4
Waste irrigation waters	16.0
Tanks	14.8
Rice fields—growing	13.1
Field channels	10.5
Seepage and spring pools	7.5
Canals, etc.	4.5
Wells	4.0
Irrigation channels	3.1

The breeding of *A. vagus* in rice fields has been noted also by Sen (1941, 1948) and others. Fallow rice fields freshly flooded by waters are preferred to growing rice fields because of more turbid water. In Assam, Muirhead Thomson (1940) found *A. vagus* breeding in muddy pools exposed to sunlight as well as in rice fields with shallow water. It had a high thermal death point, about 44°C.

Relation to Disease

Not a vector of malaria. Several thousands of dissections have been reported from India and from most other Southeast Asian countries. Except for one infected specimen with sporozoites (degenerating) found at Pattukkottai, South India, out of 6,874 dissected (Russell and Ramachandra Rao, 1940b) and two sporozoite infected specimens found in Bengal out of 10,452 dissections (Strickland *et al.*, 1933), no other infections have been found anywhere else. Lamprell (1936) gives records of over 26,195 dissections in several parts of India and Wattal (1961) of numerous other subsequent records.

Anopheles sundaicus Rodenwaldt, 1926

Type locality: Sunda Islands, Indonesia.

Type: Location unknown.

Taxonomy: Two varieties of *A. sundaicus* are: var. *flavescens* (Swellengrebel, 1924) occurring in Java (Indonesia) and

var. *torakala* Stoker and Aktoedi 1949, in Sulawesi (Celebes).

For a long time, in India, this species was known as *A. ludlowi* till King (1932) clearly brought out the differences between the two.

A. ludlowi (Theobald) is known to occur in the Philippines, China (Hainam), Taiwan, Kalimantan, and Moluccas (Indonesia).

Distribution: India, Bangladesh, Burma, 'Indochina', Thailand, Malaysia, Singapore, Indonesia, as far east as lesser Sunda Islands and south Sulawesi and China. Does not occur in Sri Lanka or the Philippines or Taiwan.

In India—West Bengal, Orissa, the coastal regions of Andhra Pradesh, and the Andamans.

There is no doubt that in India in recent years it has become very scarce and has withdrawn from many of its peripheral areas of distribution.

Distinguishing characters: The adult *A. sundaicus* is a medium sized mosquito. Its closest relatives in India are *A. subpictus* and *A. vagus,* all members of the

group *Pseudomyzomyia*. It is distinguishable from them by speckled femora and tibiae. A superficial resemblance also exists with *A. stephensi*, but the banding on the palp distinguishes the two species. In *A. sundaicus* there is only one wide apical band and not two as in *A. stephensi*. In rubbed specimens, identification can be confirmed by (1) larger preapical dark spot of wing; (2) shorter petiole of the anterior forked cell; and (3) absence of broad scales on the fossae. Males can be distinguished by the genitalia.

The larva of *A. sundaicus* is difficult to distinguish from that of *A. subpictus*, but the tri-radiate character of hair No. 5 on mesothorax seems to be a reliable character with an accuracy of about 95 per cent. In *A. subpictus* it is bi-radiate. The mesothoracic pleural hairs, post spiracular hairs and the character of the pecten used by Venhuis and others are not reliable because of their great variability. (Sen, 1949); See also Ghosh (1932); Reid (1968). Venkat Rao and Ramakrishna (1950) have stated that they were able to distinguish the salt-water breeding form and fresh-water breeding form. They found that the fresh-water form had male genitalia resembling those of *A. ludlowi* which also is a fresh-water breeder. Sundaresan and Appa Rao (1943) had made a comparative study of the larvae of *A. sundaicus* and *A. subpictus*. Their data are summarized in the Table 34.

It is, therefore, seen that the differences are not absolute, but graded and statistically useful. The best thing to do would be to rear the larvae to adults whenever possible.

Table 34. Larval characters of *A. sundaicus* compared with those of *A. subpictus*
[Sundaresan and Appa Rao (1943)]

Larval characters	Percent of times the character occurs		
	A. sundaicus	*A. subpictus* from salt-water	*A. subpictus* from fresh-water
Head band present	99.5	64.8	19.1
Hair No. 5, 3—3 or more branches	95.4	14.3	48.9
Hair No. 5, 2—2 less 3 branches	4.6	85.7	51.1
Head band present and hair No. 5, 3—3 or more branches	93.9	4.2	0.0
Setae, coarse and pigmented	89.3	20.9	0.0
Setae, fine and not pigmented	10.7	79.1	100.0
Post-spiracular hair, 7—8 branches	97.0	47.1	8.5
Post-spiracular hair, 4—5 or less branches	3.0	52.9	91.5

A. sundaicus larvae can be distinguished from *A. vagus*, by having *A. subpictus* type of clypeal hairs.

Prevalence

A. sundaicus is characteristically prevalent in coastal areas because of its predilection to breed in brackish or salty waters. It is, however, sometimes prevalent over 50 kms inland as in Orissa adapting itself to fresh water as described by Senior White.

In the Indian mainland, *A. sundaicus* (then *ludlowi*) was first found in the

marshy areas of Sunderabans of Bengal in 1912. Later it invaded the whole of the coastal area of Bengal upto a depth of 80 kms.

Senior White (1937) discovered *A. sundaicus* in Orissa. Previously efforts to detect the species in that area had been unsuccessful (Fry, 1912 quoted from Venkat Rao 1961; Sarathy, 1932). The following has been taken largely from Vènkat Rao (1961).There is reason to believe that the species invaded the Chilka Lake area for the first time after 1931 but before 1937. The fact that one W. Hunter the famous Angolo-Indian civilian and ethnographer did not refer in 1872 to malaria around Chilka Lake indicated the probable absence of *A. sundaicus*. However, it is conjectured that the species might have occurred even much earlier, even perhaps about 1815 when a severe malaria epidemic occurred in a town in south Orissa.

The invasion of Chilka lake was probably due to the freshening of the lake water due to choking of the outlet to the sea. Subsequently, *A. sundaicus* has been found all along the Orissa coast upto Visakhapatnam in Andhra Pradesh.

There seems to have been a gradual adaptation of *A. sundaicus* to fresh waters, though Covell and Pritam Singh (1942) did not find breeding of this species in several inland villages situated upto 10 kms from the shore. Venkat Rao (*loc. cit.*) refers to the species subsequently, in some of these villages, breeding locally in fresh waters. The output of adults from saline waters appears to have been much higher than from fresh water adding support to the views of the existence of two races of *A. sundaicus*. Senior White (1948) formulated the view that the fresh water form found in this area may be a new mutant race adapted to breeding in fresh waters or it may be of a primitive type which is supposed to be a fresh water species. Venkat Rao and Ramakrishna (1950) suggested alternatively that the two forms may be morphologically valid sub-species recognisable by differences in the structures of the leaflets of the phallosomes.

A question of considerable interest is whether *A. sundaicus* actually invaded India and if so when? There is no definite evidence about these matters. *A. sundaicus* has been known to exist in the neighbouring country of Burma, and also in Thailand and Indonesia as well as in the Andaman Islands much prior to its discovery in Indian mainland in 1901. Therefore, its prior existence in India particularly in coastal Bengal would not be surprising. Subsequently, however, it invaded into fresh areas like Orissa and Andhra Pradesh. Studies by Senior White and colleagues have shown that *A. sundaicus* has spread far into Andhra Pradesh near Visakhapatnam from an original focus in Bengal.

Observations since 1945 indicate that the extent of distribution of *A. sundaicus* in India has greatly dwindled, it has practically disappeared in the coastal region of Orissa and Andhra Pradesh and is now restricted to a small focus in the estuarine areas of West Bengal. That it has not completely disappeared is shown by the fact that a few adults were found as late as 1974-75 in the 24-Parganas District. Their numbers were very small, but they were just enough to carry out insecticide susceptibility tests. (Information by courtesy of Dr. S. Pattanayak).

Though recorded primarily as a species prevalent in coastal areas, its reported distribution in Burma presents some conflicting features. The map provided by

Khin-Maung-Kyi (1971) shows that the species has been found widely in several localities far inland including the hilly regions of upper Burma. This is not in consonance with the distribution given in the text where it is stated that it occurs along the coastal lowlands. But if its wide distribution is found to be correct, it does not seem to be an isolated instance because Covell (1944) had referred to the presence of *A. sundaicus* breeding in fresh waters in mountain valleys at heights of 500 to 1000 ft. above sea level in Sumatra (Indonesia). This type of distribution of the species presupposes its ability to breed in fresh waters.

In India, Venkat Rao and Ramakrishna (1950) have reported that in Chilka Lake area in Orissa and Visakhapatnam coast, *A. sundaicus* larvae have been found often in fresh waters (salinity less than 0.1%). As already stated they even suggested the possibility of existence of two races. Fresh water forms are also not unknown in other countries, such as Sumatra (Bonne Wepster and Swellengrebel, 1953) and in southern Taiwan, where the species has been found breeding in small sandy streambed pools and though to a lesser degree in rice fields (Chang *et al.*, 1950). In Borneo, the species is found as far as 900 metres inland but it seems to be associated with the salinity created by urine from domestic animals (Colless, 1948).

Adult Bionomics

Resting habits: Adults can be collected in good numbers from houses and cattlesheds. Apart from early records quoted by Christophers (1933) several observations have been made subsequently. In the Chilka lake area, out of 16,938 adults collected 52 per cent were collected in houses occupied by men only, about 25 per cent in mixed dwellings and 23 per cent in pure cattle sheds, suggesting a strong preference for human dwellings (Covell and Pritam Singh, 1942). There may be wide fluctuations in the annual densities and the places of resting may undergo changes. (Venkat Rao, and Venkat Rao *et al.*, 1942). Whether any and if so how much outdoor resting occurs in India has not been determined.

However, Reid (1968) stated that in Malaysia the adults may rest both indoors and outdoors. Khin-Maung-Kyi (1971) says that in Burma, though previous workers had always stated that the species used to rest in houses by day, it had been found that during the insecticide treatment programme the proportion resting in sprayed houses and cattle sheds was negligible in comparison with that collected biting cattle at night. Nair (1947) found that over 60 per cent rested in houses in Malaysia.

Biting habits: Very few published observations on biting activities are available. However, both Venkat Rao (*loc. cit.*) in India and Khin-Maung-Kyi (*loc. cit.*) in Burma say that from their personal experience feeding takes place mostly in the first quarter of the night. The latter author, however found that in another locality (Akyab and Kyatokpyu), the biting took place a little later between 21.00—24.00 hours. If the adults are very abundant, biting may take place throughout the night and sometimes even during daytime. In Indonesia biting (and feeding) may take place throughout the night, but the percentages caught were higher in the second

and third quarters of the night (Sundararaman *et al.*, 1957). As Reid (1968) has stated, many such observations be a misleading because of the gradual accumulation of fed females inside houses during the night. In Malaysia, Reid (*loc. cit.*) summarizing the observations made, has recorded that approximately only 29 per cent of females were captured indoors and 71 per cent outdoors biting men. When net traps were used, 11 per cent were found in the hut outside the net and 89 per cent inside the net strongly suggesting that the adults got trapped in the net and perhaps would have left the houses after feeding, if not so trapped.

Feeding habits: In Malaysia and Kampuchea, as shown by Wharton *et al.* (1964) and Eyles *et al.* (1964) respectively, attraction of *A. sundaicus* to monkeys is rather low and cattle seem to have a better attraction.

In Indonesia Toumanoff (1936) and Walch (1932) found that the percentage of females with human blood in the stomachs was 32 and 86—98 respectively. Sundararaman *et al.* (1957) found in South Java that the percentages with human blood were 51 in houses or traps and 22 out doors. But Reid (1961) found that one calf attracted 702 females in 28 nights while two men attracted only 99 during the same period (a ratio of 7:1).

Earlier studies in Burma seem to indicate that the species feeds indiscriminately on cattle or man. Macan (1947) found that more were collected biting man in the houses than cattle. October and November were the months of increased biting on man.

Very few precipitin tests have been made in India to give reliable information. Senior White in Visakhapatnam (1947) found an anthropophilic index of only 5.6 per cent among 90 specimens examined. In short its preference to man or cattle varies considerably, but its attraction towards man is certainly higher than in the case of other vectors like *A. culicifacies.*

Longevity: Studying the proportions which were multiparous and nulliparous, Moorehouse (1965) found that in Malaysia 64 per cent of females captured and dissected (total 1,221) were multiparous, while in some other important malaria vectors the parous rates were 88 per cent in *A. barbirostris*, 38—42 per cent in *A. camorenesis* and 34—40 per cent in *A. letifer.* (Reid Table 20—1968). These results suggest that *A. sundaicus* had a moderate length of life.

Under laboratory conditions, adults in cage colonies lived 2—3 weeks compared with 4—5 weeks for *A. camorenensis* and 5—9 weeks for *Culex fatigans* (Reid, 1958) in Malaysia. Early observations in that country and indicated that *A. sundaicus* could live upto 16 days. A nearly 100 per cent of those given a blood meal and maintained in cages at room temperature survived at least 16 days. Kingsbury (1931) quoted by Horsfall (1972). These are not observations of a critical nature. It should, however, be noted that the species could not have been a vector of importance, that too in warm humid coastal regions, unless it had a fairly long life. Little information on longevity exists in India. However, its densities are not of the order as those of *A. culicifacies,* suggesting either a longer life or a higher human biting rate.

Flight and dispersal: *A. sundaicus* is generally regarded as a strong flier. Venkat Rao *et al.* (1942), during their study of swarming and mating of the species, found *A. sundaicus* swarms about 3.2 kms away from the nearest breeding place. Specimens have also been collected in villages 9.6 kms away from the Chilka Lake of Orissa (Covell and Pritam Singh 1942). Covell (1944) has also pointed out to earlier records of flight of over 1.6 kms in the Andamans. Some of the other observations of interest were those by Breeman (1920)* (marked mosquitoes) 5.0 kms; Galvao (1948) (marked mosquitoes) 0.5—4.5 kms; Swellengrebel and Swellengrebel de Graff (1948)* found flights of 1.6 kms to be normal but the total numbers at 1.6 kms from a breeding place were about one half to one barrier to the flights (Covell, 1927). Passive dispersal in trains and boats over long distances has been observed by Senior White (1937) and Sen (1941) in Bengal.

Swarming and mating: Swarming and pairing of *A. subpictus* and *A. sundaicus* were observed in January 1942 by Venkat Rao *et al.* (1942) in an area close to the Chilka Lake. Swarming started at about 17.30 hours and continued till 18.00 hours when darkness prevented further observations. Each swarm contained more than one species but pairing took place only between individuals of the same species. It was observed that copulating pairs usually emerged from the group and became separated outside. The vertical movement of the swarms was about 6 feet from the ground level. The vertical movement of the swarms was about 30 cms, length about 300 cms and width about 120 cms. The dancing movements of the mosquitoes were both circular and up and down.

Larval Ecology

Breeding places: It has long been known that *A. sundaicus* is a salt water breeder. Even as far back as 1912 Christophers had noted this in the Andamans. Of course* Quoted from Horsfall (1972) he was dealing with "*A. ludlowi*" at it was then known. A lot more information has been collected since then. A tendency to breed in fresh waters has also been noticed.

The occurrence of *A. sundaicus* very close to Calcutta in the area known as 'Salt Lake' was noted as early as 1925. This area has now been completely swallowed up by the city itself. Interesting studies were made in Salt Lake area under the 'Ludlowi enquiry' under the Indian Research Fund Association (now Indian Council of Medical Research).

The major breeding places of the species are: swamps and pits along bunds, etc. containing brackish, usually stagnant water. Places in which sea water and fresh water mingle are the most suitable places. However, *A. sundaicus* has also adapted itself to breeding in fresh water.

Relation to salinity: In Bengal, Ramsay and Macdonald (1936) noted that the larvae were found in waters with between 50 and 250 parts of sodium chloride per 100,000, *i.e.* 0.05 to 0.25 per cent. In Calcutta (Salt Lake) where the species perhaps

became introduced, the salinity range was 0.16 to 0.63 per cent (average 0.164 per cent) and the pH range was 7.7 to 8.5 (average 8.2) (Sen, 1938).

Some of the reports of the salinity tolerance of *A. sundaicus* are: Iyengar (1931) 0.15 to 0.20 per cent; Iyengar (1941) 0.15 to 0.25 per cent; Jeogy (1936) 0.08 to 2.68 per cent; Covell & Pritam Singh (1942) 0.6 to 0.8 per cent; Senior White & Adhikari (1939) 0.87 per cent (optimum); but scanty breeding was found between 1.3 to 2.3 per cent (Senior White *et al.* 1947); Christophers (1912) optimum 0.4 per cent in Andamans; Covell (1932) 0.5 to 1.6 per cent. In the Chilka Lake area and Visakhapatnam area as already mentioned *A. sundaicus* is also found in fresh waters of salinity below 0.1 per cent. To sum up, it may be stated that while *A. sundaicus* breeds mostly in waters with a moderate degree of salinity, it does breed also in fresh waters, sometimes far inland where it can create a malaria problem.

In Visakhapatnam area, however, *A. sundaicus* breeds almost exclusively in fresh-water breeding places such as tanks, ponds and borrowpits (Venkat Rao 1961). In Puri Town, larvae occurred in water with salinities of less than 0.1 per cent (Panigrahi, 1942) and no larva was found in brackish waters. Had *A. sundaicus* developed a new habit or was it just a spill over, or had a new race emerged? These questions are not capable of an answer today as the species, both in brackish and in fresh waters, have disappeared from the entire Orissa and Andhra Pradesh coasts. Which waters can be called fresh or brackish is a moot question; perhaps salinity of less than 0.1 per cent may be called fresh and above that brackish.

Relation to vegetation: The association of *A. sundaicus* breeding with the growths of both higher plants and algae is well known (Sen, 1938). Vegetation is essential for the growth of larvae. Several types of algae including *Lyngbya, Anabena* and *Spirogyra* were associated with the breeding of the species (Covell and Pritam Singh, 1942) in Chilka lake area. In Indonesia, floating masses of the algae such as *Enteromorpha, Cladophora* and *Cynanophyceae* are very favourable (Swellengrebel, 1919). Walch's success in controlling the breeding of the species in the fish ponds of Java by biological control of the blue green algae is well known. Floating or submerged vegetation including forms like *Najas, Ceratophylum* and *Hydrilla* (Covell and Pritam Singh *loc. cit.*) and *Potamageton* and *Halophila* (Senior White and Adhikari 1939) are also favourable. Covell and Pritam Singh found an association with many species of algae also: *Lyngbia* and *Spirogyra* seemed to be the most important.

Organic pollution in the form of putrefying masses of vegetation seemed to provide very favourable conditions for breeding. Covell and Pritam Singh working in Chilka lake area made a statement that it was the presence or absence of putrefying masses of vegetation which was a more important factor than salinity by itself. Probably salinity had an indirect influence in controlling the growth of vegetation. Similar observations of the favourable nature of pollution had been provided by Iyengar (1944). Senior White and Adhikari (1931) had also observed that breeding was heaviest in putrefying masses of aquatic plants which were uprooted by wave

action and were floating together in masses with certain algae.

A dense cover of water hyacinth could prevent breeding of *A. sundaicus* (Iyengar, 1946). The effect was perhaps indirect, the shade preventing the growth of algae which formed the food for larvae. Contradicting Iyengar, Venkat Rao and Ramakrishna (1947) stated that in Orissa, unlike in Bengal, a water hyacinth cover did not prevent breeding of the species. It is difficult to reconcile such conflicting observations.

Relation to chemical contents of water: Among the ecological factors negatively correlated with the breeding was albuminoid ammonia content. 0.3 ppm was usually the upper limit. Very occasionally it was 0.68 ppm (Sen, 1938b).

Absorption of oxygen by the water of breeding places actually increased during the days of actual breeding. Sen (1938b) found that the relationship with alkalinity was irregular, but the breeding places of *A. sundaicus* were remarkably free of nitrites.

The studies in Chilka lake area merit special attention. *A. sundaicus* was found to be breeding mainly in the lake itself and was also found in inshore breeding places with a mixture of saline and fresh water. Tanks, ponds and jheels with heavy aquatic vegetation and situated in the vicinity of the lake were the chief inshore breeding places (Senior White and Adhikari, 1939). Covell and Pritam Singh (1942) made similar observations. Subsequently, Senior White (1947) showed that saline water was not a requisite because larvae were also found in fresh water with vegetation. It was also shown that *A. sundaicus* was exclusively a still water breeder and was not found in flowing water in nullahs, though *A. stephensi* was found in them.

Venkat Rao (1951) carried out certain experimental studies. Tanks breeding the species were drained away and the remaining seepage areas were also drained. The drains had a flow of water and *A. sundaicus* practically disappeared. In tanks which were necessary for drinking water, vegetation was eliminated periodically. He thought that converting still water to flowing water could prevent breeding of *A. sundaicus.*

The work of Walch in Indonesia (Java) of controlling breeding of *A. sundaicus* in fish ponds by a naturalistic method was interesting. The species was breeding in ponds in which fish culture was being practised. Fish needed the growth of floating algae, chiefly blue green algae, as food but the algal growth encouraged the breeding of *A. sundaicus.* Walch found a neat solution to the problem. Periodically the ponds was drained away, partly dried up, and the algae stuck to the ground and dried up. But the fish were prevented from dying by digging trenches in the pond itself. The fish found shelter in the trenches. After the proper lapse of time, the ponds were again flooded. For quite some time the algal growths would still be at the bottom, producing food for the fish but not providing shelter for the *Anopheles* larvae. Gradually the floating algae would reappear on the surface and the cycle was started again. It is not known how widely the method was adopted, but DDT certainly made it unnecessary and bothersome.

Relation to Disease

A. sundaicus is an important vector of malaria throughout its range of distribution, but with some differences in the degree of transmission. Among the earliest dissections were by Swellengrebel and Swellengrebel De Graff (1919) in Indonesia (Sumatra when they dissected 4,505 specimens and found 102 gut infections. They did not dissect the glands. They had been preceded by Barber (1918) in Malaysia who found one gut and one gland infection in 12 females dissected. They were soon followed by Breeman (1920) in Java (51 gut infections in 3813 dissected). They also did not dissect the glands. Many other workers have followed with numerous reports of gut and gland infections. The countries in which positives were found are: India, Burma, Thailand, Malaysia, Indonesia (Sumatra and Java) and Borneo (Kalimantan). (Ref. Horsfall, 1972).

In India, Iyengar (1931)dissected 836 specimens and found 71 gut and 160 gland infections (sporozoite rate above 20 per cent—a very high rate indeed). Ramsay and Macdonald (1936) had dissected 1,593 females (all glands only) and found 175 infections in Bengal. The major positive dissections in India are shown in Table 35.

Table 35. Dissections of *A. sundaicus*

	Total dissected	Gut . .	Gland . .	
Iyengar 1931	836	71	160	Bengal
Ramsay & Macdonald 1936	1,593	—	175	Bengal
Covell 1927, 1927(*a*)	98	2	1	Andamans
Sen 1938	124	2	3	Bengal
Senior White & Adhikari 1939	859	10	2	Orissa
Panigrahi 1942	617	5	7	Orissa
Covell & Pritam Singh 1942	10,714	12	51	Chilka Lake (Orissa)

Obviously the species was a vector of high efficiency in Bengal, Orissa and the Andamans.

Sporozoite rates of nearly 11 per cent and 19 per cent in Bengal are quite high by any standards. Such high rates have not been found by others. Covell and Pritam Singh in Chilka lake found sporozoite rate of only 0.47 per cent. Still it was able to maintain a fairly high degree of malaria endemicity. However, it is difficult to explain the big difference in the findings between Bengal and Orissa. According to Venkat Rao (1961*a*), Senior White *et al.* (1947) dissected 1,280 females with a total infection rate of 4.7 per cent and a sporozoite rate of 2.6 per cent. It certainly bridged the disparity a little but not enough.

Venkat Rao (*loc. cit.*) reports that the discrepancy could be sought to be explained by the hypothesis that the salt water form is comparatively harmless and

the transmission is caused by the fresh water form only. He says this was tested by eliminating all fresh water forms in a locality without touching the breeding of the salt water forms. The dissections done later showed only one gut infection (no sporozoites) among 475 dissected (average rate of 0.2 per cent). From this, he concluded that his hypothesis was supported. But it is not entirely convincing. Firstly, the dissection results from the same locality previously were not known. Secondly, no other evidence to support the existence of two forms has been adduced and thirdly, what the proportions of the two forms were even if they existed sympatrically is not known. Anyhow, it is an interesting suggestion, and has to be tested if an opportunity arises again. Was the higher infection rate due to paucity of cattle, which is not likely or was the species at that time predominantly a man biter? These are difficult questions to answer.

Covell and Pritam Singh (1942) whose dissections form the largest series in India also dissected 20,844 *A. annularis* females in the same Chilka lake area, and found only one gut infection. 2,305 females of *A. culicifacies*, 674 of *A. aconitus* and 450 of *A. varuna* were also dissected without finding any positives.

While in Orissa coast, particularly Chilka lake area, the role of *A. sundaicus* is established, Senior White (1947) thought that in central parts of the coast, it was not *A. sundaicus* which was the vector but *A. stephensi*. In Puri town, *A. annularis* was a vector secondary to *A. sundaicus*. (Panigrahi *loc. cit.*)

Did *A. sundaicus* ever reach Madras in the past? A very interesting record is that by Hodgson (1914) when he stated that *A. ludlowi* (=*sundaicus*) had been found infected by one Horne in Madras City in 1913 when there was a serious outbreak of malaria there. Though *A. culicifacies* and *A. stephensi* were also found they were not found infected. Hodgson noted that according to local opinion malaria was a new feature in the health of the city. Covell and Pritam Singh (1942) thought that the epidemic could have been due to an invasion of *A. sundaicus*. However, *A. sundaicus* has not been found in Madras in any of the many surveys carried out in recent years. The present malaria prevalence in Madras City is primarily due to *A. stephensi*.

In Burma, no infected specimens have been found. According to Khin-Maung-Kyi (1971) Lalor in 1912 dissected 400 females and Feegrade in 1912 dissected 58 specimens with negative results. In 1952 Macan dissected 370. Though no infections have been reported in Burma, it is suggested to be a vector in some coastal areas purely on epidemiological grounds. In Thailand and Malaysia it is a weak vector; and only in Indonesia it is an important malaria vector.

Malaria zoonosis of simian origin in Andaman and Nicobar Islands. Kalra (1980) has now reported that in 1979 in the Greater Nicobar Islands, symptoms resembling those of *vivax* malaria were occurring among the inhabitants. The blood smears showed a malaria parasite closely resembling *P. cynomolgi*. *Macaca umbrosus*, the crab eating monkey, is the only monkey in the islands and two of the 13 monkeys examined were found to harbour the same parasites. Kalra postulates that certain

changes in the behaviour of *A. sundaicus* have resulted in its readily feeding on monkeys. *A. sundaicus* is already known to be susceptible to strains of *P. cynomolgi bastianelli.* It was likely that it transferred the parasites from monkey to man and therefore paved the way for a large scale development of malaria zoonosis.

A. sundaicus is susceptible to strains of *Plasmodium cynomolgi.* (Bennett *et al.* 1966). See under "Additional Notes" at the end of this Chapter for monkey malaria zoonosis in the Andamans.

Anopheles multicolor Cambouli n, 1902

Type locality: Isthmus of Suez, Egypt.

Type: Faculty of Medicine, University of Paris, Paris, France. (Now believed to be non-existent).

Taxonomy: No recognized varieties. A form known as *A. nigrifasciatus* Theobald, in North Western parts of Pakistan is now regarded as a synonym. *A. nigrifasciatus* of Davys (1912) is actually *A. superpictus.* Two other synonyms are known, but they do not occur in the Oriental region.

Distinguishing characters: It is comparatively a large sized mosquito. Wing length from 3.6 to 4.7 mm. It shares along with *A. turkhudi* the character of having a black tip to the palps. These are the only two species with this feature in India.

Distribution: North Africa, Cyprus, West Asia, Afghanistan, Pakistan, India. In India: Confirmed specimens have been collected in Panchmahals District of Gujarat (Viswanathan 1950), Very rare.

Bionomics: No information exists on its bionomics in India. Most of what we know about it is from West Asian countries and Egypt where it is highly prevalent. It is known to readily enter houses and bite man. The species is known to fly long distances when on the wing and Christophers (1933) mentions that it was found at a distance of 13 kms (8 miles) from the closest possible breeding places. The larvae were found breeding in pools and disused wells and could tolerate quite high degrees of salinity upto 6 per cent. It is a desert species.

Relation to disease: No records of dissections in India but in other countries it is regarded as a vector as in the cases in the Sahara desert on epidemiological grounds. Earlier records quoted by Christophers (1933) indicate that it may be the only species of Anopheles in such localities, but natural infections do not seem to have been reported (See Horsfall, 1972). The species has been experimentally infected with *P. falciparum.*

Anopheles turkhudi Liston, 1901

Type locality: Ellichpur in Vidarbha area of Maharashtra State (Formerly Central Provinces).

Type: British Museum of Natural History, London.

Taxonomy: There is only one recognised variety, *telamali* Saliternik and Theobald, 1942, occurring in Israel. The forms *azriki, flaviceps,* and *persicus* are now regarded as synonyms by Stone *et al.* (1959). In earlier years some misidentifications have occurred of *A. multicolor* and *A. hispaniola* as *A. turkhudi.* Strangely the type of male *A. culicifacies* deposited by Giles is stated to be a specimen of *A. turkhudi.*

Distinguishing characters: The adult is comparatively of a large size though not so large as *A. "hyrcanus", A. barbirostris* etc. The species of Oriental anophelines resembling *A. turkhudi* most are *A. multicolor* and *A. superpictus. A. turkhudi* and *A. multicolor* can be distinguished from *A. superpictus* by having the tip of the female palp dark. Even in the male the tip is quite dark in *multicolor* and predominantly dark in *A. turkhudi* quite unlike any other Indian anophelines.

Characters which distinguish *A. turkhudi* and *A. multicolor*

	turkhudi	*multicolor*
The fossae at side of mesonotum:	Devoid of scales	Covered with broad scales.
Extreme base of costa:	Dark	Bright
Upper part of sternopleuron:	No or few scales.	Well provided with scales.
Vein 6:	One long distal spot and a short basal spot.	Three dark spots.
Dark tufts of spines at tip of female palp:	Less prominent.	More prominent.
Male palp:	Tip usually with a few pale scales.	

Distribution: Adult, a large sized mosquito extensively prevalent in countries west of India upto Morocco. India, Pakistan, Afghanistan, Israel, Saudi Arabia, Yemen, Aden, Sudan, Somaliland, Egypt (Sinai), Morocco, Eritrea, Ethiopia.

In India: Maharashtra, Gujarat, Karnataka, Madhya Pradesh, and Rajasthan. Recently one specimen found in Himachal Pradesh (Ramachandra Rao *et al.* 1973). May occur in Punjab and Haryana also.

Ecology/Bionomics: Being a rather uncommon species its ecology is comparatively little known. Adults are collected in houses. Large numbers of *A. turkhudi* have been collected in the Deccan region of Maharashtra in the districts of Poona, Sholapur, Nasik, Jalgaon (East Khandesh) and Dhule (West Khandesh) in houses and cattlesheds at altitudes ranging between 500 and 1,000 metres. Christophers has mentioned that this species was found at altitudes of 1,200 to 1,800 metres, and adults have been captured at heights of about 2,800 in Pakistan. Mulligan and Baily in Quetta (Pakistan) (1936) had found that the species would fly upto one kilometre.

Larvae are found in shallow pools on the ground among green algae. Frequent

replacement of water seems to be essential as it occurs in pools in river beds. In Palestine also the larvae are stated to occur in similar pools connected with streams but with rich growths of green algae. In the Deccan region, the larvae were found in similar breeding places in association with *culicifacies* and *fluviatilis* larvae (Present author, unpublished).

No information is available on other aspects of bionomics.

Relation to disease: Not many dissections have been carried out. Barber and Rice (1938) dissected 10 in Poona district; Soman (1945) dissected 16 in Bombay Deccan; Bhaskar Rao *et al.* (1946) dissected 10 in the Bellary district of Karnataka; DeBurca and Jacob dissected 15 in North west region of Pakistan and Subramanian and Dikshit dissected five in Khandwa in Madhya Pradesh. None of these was positive. A larger series of dissections made so far is by Bhatt, who dissected 417 specimens in Nasik District of Maharashtra with one sporozoite positive (Bhatt, 1949). However, they were not likely to be sporozoites of human malaria but probably were of bird plasmodia. The sporozoites were found to have an average length of 8.8 microns (ranging from 8.4 to 9.8 microns) as against an average of 12.3 microns (ranging from 11.2 to 13.00 microns) of sporozoites found in a specimen of *A. culicifacies* from the same area.

No information in relation to animal plasmodia or filariasis has been traced.

Anopheles annularis Van der Wulp, 1884

Type locality: Mount Ardjoeno, Java (Indonesia).

Type: State Museum of Natural History, Leiden, Netherlands.

For a long time this species was known in India as *A. fuliginosus* Giles, 1900 (Type locality: Calcutta) till Christophers and Barraud (1931) recognised it as *A. annularis* already described by Van der Wulp in 1884.

Synonyms are:

fuliginosus Giles, 1900 India, *jamesii* Donitz, 1901 Sumatra, Indonesia, *lineata* Ludlow, 1908 Philippines, *adiei* James and Liston 1911 India, and *nagpori* James and Liston 1904 India.

The two latter are now regarded as melanic forms.

Distribution: Oriental region from Afghanistan and Pakistan through India to "Indochina" and the Philippines, south to Sri Lanka and north to China.

In India: It occurs in every zone including Kashmir, but does not occur in the Andamans and Lakshadweep.

Distinguishing characters; Adult a medium sized mosquito. In common with the other members of the group consisting of *pallidus* and *philippinensis,* it has the following characters:

Femora and tibiae not speckled; segments 3,4 & 5 of hind tarsi continuously white and broad white band at apex of segment 1.

Differs from others by having wing vein 5 extensively dark and having a distinct dark spot at least in the middle. *A. annularis* is a black mosquito darker than the two other related species.

Larva: Brush-like outer clypeal hairs; sutural hair simple or bifid distally; palmate hair on abdominal segment 1 moderately developed.
(See key under *A. philippinensis*).

The species is well known for the melanic variations in adult markings, in different seasons (Covell, 1927). Wattal *et al.* (1960) have now described the details of these variations and one should guard against jumping to conclusions that a new variety has been found on the basis of such variations.

Prevalence

A. annularis is a common species in most areas of the eastern and south-eastern parts of India, but becomes scarcer towards the west. It occurs throughout the year but with greater abundance in May and June both in Bengal and southern India. Though one would at the outset think that this is due to the high concentration of water with vegetation and plankton providing optimum opportunities for intense breeding, it is not borne out by the observation that the decline in abundance sometimes commences even before the dilution of the water of the habitats commences in June-July. However, silt brought in by fresh river or irrigation water may be an important inhibiting factor. In Orissa, however, autumnal months seemed to be the season of the highest abundance, as shown by Senior White and colleagues. Such local differences cannot but be expected. The species, though abundant in the low lying areas or plains of India, has also been found at altitudes of about 2,400 metres in the Himalayas. The most westerly records in West Pakistan is the Rohat Kangu valley 80-100 kms west of the Indus River. It has been found in Afghanistan both in the low lying Laghman province of eastern Afghanistan (Ramachandra Rao, 1951) and in the Kabul province at elevation 1,000 to 2,000 metres, (Iyengar, 1955).

Adult Bionomics

Day-time resting habits: Adults of *A. annularis* can be collected easily in fair numbers in houses and cattle-sheds during day-time. In southern India they were collected in human, mixed and animal dwellings at 0.8, 0.6 and 0.3 per man-hour respectively showing a slight preponderance in cattle sheds (Russell and Ramachandra Rao, 1941). Similar observations have been made all over India. Senior White *et al.* (1934) observed that cattle sheds yielded about 30 per cent more than human dwellings in Orissa. However, in Delhi there was no significant difference in the numbers collected in houses and cattle sheds (Bhatia *et al.*, 1958).

Little information exists on the outdoor resting habits in India though presumably it occurs, but in a small degree. Senior White (1946) reported collecting only one specimen resting on the bank of a stream in Madhya Pradesh in an area where the species is not prevalent in large numbers. Adults of *A. annularis* have also been collected in small numbers in bushes, grass, etc. away from habitations.

In Burma, normally the adults are collected in houses. But according to Khin-Maung-Kyi (1971) early investigators like Lalor and Feegrade had noted that during many surveys no adults were collected in houses though larval breeding was

going on. In the DDT era, the adults were not found in houses at all, but mostly found resting in bushes and undergrowth close to cattle sheds. Even as early as 1949. Fox had shown that in certain foot-hill areas of Mandalay-Maymao, though many *A. annularis* were collected in night collections none was found resting in houses during day. Observations in Malaysia are similar to those in India.

In two villages near Lahore Reisen *et al.* (1976) found *A. annularis* adults resting in outdoor shelters. The per man-hour figures for the adults collected in different types of places compared to cattle sheds were:

	Females	Males	
Cattle sheds	2.2	0.0	
Rice fields	3.0	1.0	
Water hyacinth	1.0	0.0	Outdoors
Millet fields	1.0	0.0	

The only other anopheline found resting outdoors was *A. subpictus* but the proportions resting in cattle sheds and in the open (under water hyacinth) were 37.2 and 5.0 to 16.0.

Reisen, Aslamkhan and others (1976) made hand collections of mosquitoes found indoors at various times of day and night. They found a density of only 2 per man-hour in collections at 09.00 hours, 0.5 at 12.00 hours and 0.0 at 15.00 hours while similar figures for *A. culicifacies* were 32.0, 57.5 and 48.5 respectively. This indicates that *A. annularis,* though it occurs in large numbers, is not abundant in houses during day-time.

Flight and dispersal: Direct observations on this aspect of bionomics in India are lacking. As with other Indian malaria vectors, 0.8 kms is regarded as the usual effective flight range. Very early records of flights made in 1902 and 1903 by Christophers and Sinton and by James respectively had also suggested flights upto 800 m.

Biting habits: There are very few observations on the nocturnal activities of *A. annularis* in India. Venkat Rao (1961) states that he had made an observation in Orissa State that the adults entered a dwelling house (a student's hostel) in the second quarter of the night. However, in cattle sheds the species was found to bite throughout the night, in greater numbers before midnight.

In Pakistan, it is eight times more attracted to cows than to men and feeds on cattle upto 02.00 hours. In a study in Changa Manga National Forest near Lahore, Aslamkhan and Salman (1969) found 55 adults biting in the first quarter of the night, 36 in the second quarter, 21 in the third quarter and none in the fourth quarter of the night. These indicate that the tendency is to bite earlier in the night. Studies in Pakistan by Reisen, Aslamkhan and others have shown that the biting rhythm of *A. annularis* was mainly crepuscular and most of the biting was over between dusk and 20.30 hours. There was a slight shift upto mid-night in the warmer months, but practically no biting occurred after mid-night. They collected over 2,560 adults in their year-long studies carried out in 1976.

Host preference: The species has been regarded as predominantly a cattle biter,

though it is not averse to biting on man. Precipitin tests have been scanty. Afridi (1939) tested 37 stomach bloods and found that all had fed on cattle. In Orissa coastal plains and east Central India (parts of Madhya Pradesh), Senior White (1947) found anthropophilic indices of 1.3 to 1.8 per cent respectively out of 834 and 614 stomach bloods examined respectively. Precipitin tests in other countries, such as Malaysia and Indonesia, are also very few presumably because the species occurs in small numbers there. But wherever observations have been made it is predominantly a cattle feeder. In Burma it has been reported that in the absence of cattle, the species will bite man readily but when cattle are present, it predominantly feeds on them.

Swarming and mating: Though the probability of swarming occurring in Indian anophelines had been suspected earlier, the first description of swarming of anophelines in India was made by Ramachandra Rao and Russell (1938). *A. annularis* males were found swarming regularly in the evenings just after sunset in a tennis court, close to a tank where the species was breeding in Pattukkottai. The swarming commenced between 18.30 hours and 18.40 hours in March. Quite dramatically the mosquitoes would come from all directions and within a minute of their first appearance definite groups hovering about and performing swarming movements would be formed. The height above ground was generally about 6-7 feet, when above the heads of persons standing or as low as 3 feet above ground on persons sitting on deck chairs. Using a light meter of high sensitivity, it was found that swarming generally started when the light was 1.6 to 1.9 foot candies. When the swarming ended the light was too low to be measured, even with the sensitive apparatus used. The fall in light intensity was very rapid at that latitude. From over 500 FC the upper limit of the meter, the light intensity dropped to 2.0 FC in about half an hour and from 2.0 FC to 0.02 FC it took only minutes and the swarming occurred during the later period.

There was always a good breeze when swarming occurred and on one occasion when light rain had fallen just prior to the usual swarming time no swarming occurred.

The swarms were composed mainly of males. Females entered the swarms, grabbed the males and left the swarms. They included not only freshly hatched specimens but also many older ones. The swarming movement consisted of up and down movements ranging from a few cms to one metre and a forward and backward movement upto 30 cms. The number of mosquitoes in a swarm ranged from two or three to over a hundred. During these movements the individual mosquitoes always faced against wind. (For comparison with natural swarming of *A. culicifacies* in Pakistan, see under that species). *A. subpictus*/*A. vagus* males were also found on one occasion and a single specimen of female of *A. culicifacies*.

Culex species were also found swarming quite frequently at several places. Not only were the *Culex* species found swarming at dusk but definite swarms were also observed at dawn when the light intensity was about the same as in the evenings. It is interesting to note that Mayflies (Ephemeroptera) also formed large swarms both at dusk and dawn.

Venkat Rao *et al.* (1942) made further observations in the Chilka lake area where both *A. sundaicus* and *A. subpictus* were found to swarm. Subsequently, Venkat Rao *et al.* (1961) recorded still further observations on *A. annularis* made in 1946. Much breeding of *A. annularis* was occurring in the Chilka lake itself. The swarming was observed at about 17.30 hours at 2 m above the ground close to the lake. Copulating pairs were seen. An interesting finding was that an almost continuous line of swarms was found upto 800 m from the lake. Sometimes the swarms of *A. subpictus* and *A. sundaicus* occurred in an elevated place of land about 6 m above the lake level and 3.2 km away from the lake. But the swarms of *A. annularis* were not more than 1.6 m above the level of and much closer to the lake.

In Pakistan, Reisen *et al.* (1977) made an extensive study of swarming and mating of some mosquitoes in nature *A. annularis* was one of them. They found swarms of the species occurring a little earlier than in South India. When observations were made against certain markers with white and black background, *A. annularis* swarms occurred over both, but there were 19 swarms over white surface while only one swarm was found over black. The markers were of the "clothes tree" type and were 1.25 m high with 0.5 m wide parallel to the ground. Dry black and white clothes were draped over the cross pieces.

Under laboratory conditions mating has been found to take place in cages (60x30x30 cms). When wild females were examined, nearly all had mated whether they had previously fed or not (Roy, 1940).

Longevity: No positive information is available regarding longevity in nature. In cages females have been found to survive upto 16 days at relative humidity of 38 per cent (Sinton and Shute, 1958) or upto 27 days (Kingsbury 1935, quoted by Horsfall, 1972).

Gonotrophic dissociation: Venkat Rao (1961) has emphasised the importance of gonotrophic dissociation or discordance in mosquitoes particularly in *A. annularis* on malaria epidemiology. Gonotrophic discordance is the phenomenon which is characterized by the blood feeding and maturation of ova not keeping pace with each other. There may be more than one blood feed before the eggs mature and are laid. Gonotrophic dissociation is the phenomenon in which there is no growth and maturation of eggs at all and the females continue to take blood meals without egg development. He has found evidence for occurrence of both phenomena in Orissa. The repeated blood feedings tend to enhance the probabilities of transmission of malaria in the locality, and in gonotrophic dissociation the lack of any necessity for the females to leave the shelters reduce their exposure to hazards of an outdoor life, prolonging longevity and therefore leading to increasing chances of transmission. Gonotrophic dissociation is somewhat like the hibernation of anophelines in cold climates of Europe, but it is not true hibernation because the anophelines continue to feed. However, they also accumulate a transitory fat body.

Larval Ecology

A. annularis is predominantly a breeder in still waters with abundant vegetation. Therefore, tanks and ponds, borrowpits close to canals, margins of weed-grown

rivers, etc. are the well known places of breeding. Rice fields are not generally favourable except in a short season. However Sen (1948) recorded *A. annularis* breeding in rice fields in association with *A. "hyrcanus"* both in the seedling stage and in the later stages of rice growth.

Sen (1941) made a special study of the types of vegetation favourable to different species of *Anopheles* in Bengal, and found with respect to *A. annularis* that, waters containing species of *Hydrilla, Verticillata* and *Ceratophyllum* were most favourable.

In their extensive studies places of anophelines in southern India Russell and Ramachandra Rao (1941) found that out of 6,780 larvae of the species collected 6,047 were found in tanks. Out of 1,523 tanks searched 523 were found to be breeding this species. It was interesting that the closely related *A. pallidus* was predominantly a breeder in rice fields. 6,352 larvae out of 7,857 *A. pallidus* collected were found in rice fields. These figures showed the very clear differences in preference in breeding even among closely related species. *A. jamesii,* however, resembled *A. annularis* more than *A. pallidus.*

The larvae of *A. annularis* were also found in several other types of breeding places, particularly waste irrigation water in which it was found 108 times out of 668, and borrow pits 39 times out of 396. In rice fields it was scarce, found only in 10 fallow fields out of 336 and 6 growing fields out of 779. It was found in wells only 13 times out of 1,240. The waste irrigation water in which the species was abundant mainly consisted of large numbers of ground pools or low lying, pond-like pools close to irrigation channels and heavy growths of vegetation. In the present author's experience the aquatic plants which were most abundant in favourable breeding places were species of *Nelumbium, Vallisneria, Elodea, Ceratophyllum* and some *Allismaceae.*

In the laboratory, ice water with *Hydrilla* was most attractive for egg-laying by *A. annularis* (Biswas *et al.* 1980).

Among algae *Chara* did not seem to affect breeding of *A. annularis* in any manner. The other algae found were *Spirogyra* and *Pithophora.* The blue green algae which was common during the breeding season was *Lyngbyia.* The water was also generally clear though it became a little turbid soon after the irrigation water was let into the area because of silt. Months of April and May were the period for most intense breeding of *A. annularis.* The number of larvae collected in different months were:

January	676	Cool months
February	534	"
March	1,615	"
April	2,523	Dry season
May	2,015	"
June	1,913	"
July	834	Irrigation season
August	590	"

September	537	"
October	521	"
November	439	N.E. Monsoon
December	881	"

In Orissa, Senior White *et al.* (1943) found that tanks, borrow pits and rice fields were the major breeding places. They recognized many aquatic weeds which were favourable and unfavourable. *Hydrilla, Ceratophyllum, Nymphea, Nelumbium* and *Salvinia* species were found closely associated with the breeding of *A. annularis*. The species which were unfavourable were *Lemna, Wolffia* and *Eichhornia*. In rice fields the breeding was associated with the green algae, *Spirogyra*.

Water hyacinth was found unfavourable to the species in Bengal also (Sen, 1941). Probably the effect was indirect through the adverse effect on the growth of plankton.

A. annularis is a species which certainly prefers still waters. Occasionally it has been taken in flowing water, but the probability is that along the grassy margins of the streams the water was really still, though replaced regularly. Occasionally the species has also been found in saline waters as in Chilka Lake (Venkat Rao and Ramakrishna, 1947) and in Indonesia.

Relation to Disease

Though the species occurs very extensively in India, sometimes in large numbers, it is not regarded as a major malaria vector, but it has certainly importance as a local vector in several localities, particularly in coastal Orissa, Bihar, Bengal and Assam. Stray infected specimens have also been reported from Punjab (Ferozpore) and West Uttar Pradesh (Jhansi) (Pritam Singh, 1955).

The dissection records are quite extensive and include many negative reports. In evaluating the importance of the species one cannot always go by dissection results alone, but try to have a total epidemiological picture.

In Orissa, Bengal and Assam, where it is regarded as a vector of some importance, its role is often overshadowed by the importance of other species like *A. sundaicus* in Orissa and *A. philippinensis* in Bengal and Assam. Whether it is an exclusive vector in any area needs further critical evaluation of the previous data.

Density of adults is as important as the infection rates. In Orissa coast, where it is certainly a vector of some importance in a locality, it is effective because of high densities; for example upto 64 per man-hour in houses and 158 per man-hour in cowsheds (Senior White *et al.*, 1943). They dissected over 9,000 females and found a sporozoite rate of only 0.08 per cent. This is almost similar to the low infection rates found for *A. culicifacies* in many places. The latter species transmits malaria of a moderate degree even with infection rates as low as 0.1 per cent. The role of *A. annularis* in Orissa Coast seems to be similar. In Bengal Iyenger (1948) records dissections of over 1,700 with negative results. A few of the major series of positive dissections are given below:

	Number dissected	Positive Gut	Positive Gland	Total positives
Covell (1927), Bengal	2,511	3	3	7
Senior White and Adhikari (1940), Bengal	1,999	2	—	2
Iyengar (1940), Bengal	5,155	1	1	1
Viswanathan *et al.* (1941), Assam	7,481	4	7	—
Anderson and Viswanathan (1941), Assam (Cumulative)	16,760	2	6	?
Panigrahi (1942), Puri Town (Orissa)	9,096	5	7	?
Senior White (1943), Orissa	9,183	14	7	?

A. annularis has also been found infected in nature in the outer Terai region of Nepal in two localities viz. Nawal parasi District of Lumbini Anchal and Parsa District of Narayani Anchal (Mr. S.L. Shrestha, personal communication; 1979) indicating that the species has a much wider sphere of influence than was believed earlier.

Infected specimens have also been found in Burma, Malaysia, Indonesia and China but in low degrees.

Venkat Rao (1949) has pointed out that *A. annularis* is a vector in Orissa in three districts, Balasore, Cuttack and Puri only. He has discussed the historic reasons why this area has become endemic for malaria. These are the cultural centres in Orissa and have the most intense cultivation.

Continuing the investigations of Knowles and Basu (1943) on *A. stephensi,* Basu (1943) made similar studies on *A. annularis.* 12,851 laboratory bred *A. annularis* were fed on 129 gametocyte carriers, (*falciparum* 62; *vivax* 52; *malariae* 15) and exposed to 34 different combinations of temperature and relative humidity. Relative humidity did not seem to have any direct influence on the development of malaria parasites. Of course, it had an indirect influence on the life of the mosquitoe at higher temperature and higher humidities. At 37.8°C with 50 to 100 per cent relative humidity no infection was seen as the mosquitoes did not survive long enough. However, at 15.6°C to 32.2°C with similar relative humidities, gut and gland infections with *vivax* and *falciparum* were observed. Infectivity rate increased as the temperature rose whereas higher humidity did not result in any increased infectivity. At 10°C no infections were observed with *vivax* and *malariae,* but a few gut infections occurred with *falciparum.*

Sometimes organisms resembling sporozoites are found in the salivary glands but on staining they do not take up the characteristic stains of sporozoites of malaria plasmodia. Jaswant Singh *et al.* (1951) found such bodies in females of *A. annularis* while working on transmission studies on *P. berghei.* The bodies were considered to be a fungus (probably referable to *Chytridinae* according to late H.N. Ray, the noted protozoologist, who was consulted).

The bodies were not present in freshly hatched females but obviously developed later. Unfortunately no males were dissected.

Monkey malaria: Jaswant Singh *et al.* (1949) were able to infect both *A. annularis* and *A. stephensi* with *P. knowlesi.* Out of 123 *A. annularis* dissected, two gut and five gland infections were found. The figures for *A. stephensi* were: dissected 317, positive seven gut and seven gland infections. Experimental transmission to fresh *Macaca radiata* monkeys was obtained. None of the fed *A. subpictus* and *Culex bitaeniorhychus* was infected. The role of *A. annularis* in nature is, however, not determined.

Filariasis: *A. annularis* has been found infected experimentally with *W. bancrofti* (Das, 1976). Low natural infections with *B. malayi* (0.8 per cent) have been recorded from Calcutta (Ghosh and Hati, 1966) but are not regarded to be significant.

Anopheles jamesii Theobald, 1901

Type locality: Quilon, Kerala State, India.

Type: British Museum.

Taxonomy: This name has quite often been misapplied to other species and names of the other species applied to *A. jamesii.* The species with which it has been most confused are *A. annularis, A. splendidus* and *A. ramsayi.* The name has been re-spelt as *jamesii* by Stone, *et al.* (1958) and Knight and Stone (1977) instead of *jamesi* as used by Christophers. Reid (1968) also uses the spelling *jamesii.*

Distribution: India, Nepal, Bangladesh, Burma, 'Indo-china', China and Sri Lanka.

In India: Assam, West Bengal, Orissa, Madhya Pradesh, Goa, Gujarat and all southern states, but not found in north-western regions; absent in the Andamans and Lakshadweep.

Distinguishing characters: Adult: a medium sized mosquito, characterized by golden yellow scales on the apical part of dorsum of 7th and 8th abdominal segments. From *A. ramsayi,* it is distinguished by lighter wings; vein 5 usually without a dark mark at the fork; the under surface of femur, tibia and first tarsal segments not conspicuously pale.

From *A. splendidus* it differs by having only one broad apical band on palp which is unspeckled; wings lighter, speckling on legs less prominent; fossae with scales.

Bionomics/Ecology

Being comparatively a scarce species and not being a malaria vector, knowledge about its bionomics/ecology is meagre.

Adults are found resting inside houses and cattle sheds during day time. In south India adults are found in almost equal numbers in houses, mixed dwellings and cattle sheds (Russell and Ramachandra Rao, 1941), but the density is very low (average 0.2 per man-hour). January to May was the season of greatest prevalence though it occurs throughout the year.

The species appears to be comparatively more abundant in the Western Ghats. 2,985 adults were collected in North Kanara District out of a total of 42,960 specimens of all species (Viswanathan, 1950). Jaswant Singh and Jacob (1944) working in the same district and Covell and Harbhagwan (1958) in Wynaad in Kerala collected many adults. However, Brooke Worth (1950) in the same Western Ghats in old Mysore State, has found the species not very abundant in houses. Of 99 adults collected in a preliminary experiment, he found only 4 in day-time hand collections, 56 during night hand collections and 38 in traps at night.

Larvae prefer to breed mainly in tanks, fallow and growing rice fields, but they occur in all types of breeding places.

In South India, in Thanjavur District, *A. jamesii* preferred to breed in tanks. It was occasionally taken in wells and in waste irrigation water. Like *annularis*, it preferred waters of tanks which had growths of vegetation. It was closely associated with *A. annularis, A. culicifacies* and *A. subpictus.*

In Bengal, it is found in small numbers along with *A. annularis* breeding in tanks covered with water hyacinth (Senior White, 1946). Sen (1938, 1948), in his study of vegetation in relation to *Anopheles* breeding in Bengal, did not find the species either in rice fields or in ponds in association with several species of aquatic vegetation, though eleven other anopheline species were found breeding.

Relation to Disease

No natural infections have been found of malaria plasmodia in dissections of nearly 4,000 adults in Kerala, Tamil Nadu, North Kanara, Andhra Pradesh, Bengal, Assam, Bihar and Mysore. Wattal (1961) has given a very comprehensive list of dissections to which may be added 406 dissected with negative results in North Kanara (Viswanathan, 1950).

Anopheles karwari (James), 1903

Type locality: Karwar, North Kanara District, Karnataka State (formerly in Bombay State), India.

Type: British Museum.

Taxonomy: There is one synonym *nigrans* Stanton (1912) nom. nova. in error. The female type of *A. maculatus* now existing is regarded as *A. karwari.* Stanton (1912) therefore gave a new name *A. nigrans* for the species. But for reasons explained by Christophers (1933) viz. that the description given by Theobald for *A. maculatus* was correct, the name of *A. karwari* stands though its nominated type female was not correct. *A. karwari* is a distinct species with a correctly nominated type. (See also under *A. maculatus*).

Distribution: Wide distribution: extends from India, excluding north west regions to Philippines and north to China (Hong Kong) and in Borneo and New Guinea. Occurs in Sri Lanka.

In India: In many localities, pertaining to eastern, central and southern States including Karnataka, Maharashtra and Goa. But not in western parts, Rajasthan,

Punjab and beyond. Not occurring in the Andamans and Lakshadweep.

Distinguishing characters: Adult of medium size; palpi with 4 pale bands, the apical pale band, a subapical long band, a third narrow band separated by narrow dark band and a very narrow band at base. The closest relatives are *maculatus* and *majidi* from both of which it can be easily distinguished, from the former by unspeckled legs and from both by the palpal bandings.

Larva: See key.

Prevalence, Bionomics and Ecology

A comparatively uncommon species. There were many records of its occurrence in houses and cattle sheds during daytime. Also it has been found resting outdoors in Malaysia. In the Assam area, apparently, it did occur in small numbers.

It has been found to feed on man. Ramsay *et al.* (1936) did precipitin tests on 311 adults and found 16.4 per cent with human blood. There are many records of human biting in Malaysia (See Horsfall). Commenting on the scarcity of the species in the Malnad area of the Western Ghats of Karnataka, Brooke Worth (1953) speculated that perhaps it has some specialized requirements which are not found in that area, though the types of breeding places needed are all there. In Burma, it is regarded as a rare and pre-monsoon species.

Very little is known of the aquatic biology of the species. It breeds in several types of breeding places mainly in springs, seepages and slow running streams. Also it has been found in ponds. In his excellent summary, Covell (1944) stated that the breeding places were "clear shaded streams, spring pools, drains, seepages and swamps." Seepages seem to be preferred.

Relation to Disease

Many dissections have been reported, generally in small numbers, but the largest series of dissections was in Cachar (Assam) by Ramsay (1930) when 9,252 specimens were dissected, all negative. In the same area, Strickland (1929) dissected 1,697 and found one gut positive. See list by Wattal (1961). To Wattal's list must be added 10 dissections in North Kanara.

The species is not regarded as a vector anywhere else in Southeast Asia though a few scattered infected specimens have been found. Infected specimens have also been found in New Guinea, where perhaps it was accidentally introduced. It cannot be ignored as a non-vector until field studies are made in areas where it is very prevalent.

Anopheles maculatus Theobald, 1901 and var. *willmorei* (James), 1903

Type locality: Hong Kong.

Type: British Museum. The male type is stated to be correct but the female type is stated to be a specimen of *A. karwari*.

Taxonomy: There are three synonyms: *pseudowillmorei* Theobald, 1910 from West Bengal; *dravidicus* Christophers, 1924 from Nilgiri Hills, Tamil Nadu; and *hanabusai* Yamada, 1925 from Taiwan and one recognised variety, var. *willmorei* (James), 1903.

Type locality: Kashmir and type in British Museum. (See Christophers, 1933, Stone *et al.* 1959 and Knight and Stone, 1977).

The variety *willmorei* was first spelt *willmori,* but has been amended correctly according to the rules of nomenclature to *willmorei.* It was first described in 1903 as *Nyssorhynchus willmori* by James with a specific status. Later Alcock (1913) reduced it to a varietal status which was followed by Christophers (1933). But Stone, *et al.* (1959) gave it a subspecies status. However, Knight and Stone (1977) have again regarded it as a variety. It has two synonyms:

dudgeoni Theobald, 1907—Type locality: Kangra Valley, Himachal Pradesh.

indica Theobald, 1907—Type locality: Dehra Dun, U.P.

Distinguishing characters: Adult medium sized. The speckled legs combined with the all white segment 5 of the hind tarsi should distinguish it from all closely related forms. There are three pale bands on the female palpi, two broad apical ones and a narrow basal one. In *A. karwari* and *A. majidi* which somewhat resemble it in the banding on the legs, the legs are not speckled. In *A. theobaldi,* in which the legs are speckled the last two tarsal segments (4 and 5) of hind legs are continuously white.

Larva: See key.

The variety differs from the type form by the following characters:— Profuse scaling on the abdomen, with a well marked patch of scales on abdominal segment II. Scales broad.

Reid, *et al.* (1966) did not find any real morphological differences between forms in India and in Malaysia, though some minor differences were found, such as shorter sutural hairs, indicating the possibility of a mere geographical variety.

A completely unspotted strain with dark wings and legs quite unlike any other member of Cellia, has recently been detected and reared to four generations in laboratory colonies in Thailand but the strain was lost (Sucharit, *et al.* 1979).

Distribution: Very widely distributed in the Oriental Region, Pakistan to Indonesia.

In India it occurs in all zones; more prevalent in the east. It occurs in the Andamans but not in Lakshadweep.

The variety *willmorei* occurs in India, Pakistan, Nepal and Burma. In India varietal form occurs mainly in the Himalayas and eastern India.

Prevalence

Though it occurs in many zones in India, it is characteristically a hill or foot-hill species. The type form occurs widely all over India, but the variety occurs only in the Himalayan region. The highest elevation at which it has been collected appears to be at Chitral (Pakistan). Ramachandra Rao *et al.* (1973) found the variety *willmorei* in all districts of the Himalayan region in Uttar Pradesh and Bengal and in

most districts in Himachal Pradesh and in Doda, Udhampur and Baramula Districts of Kashmir. *A. maculatus* type was also found in their studies. The altitudes at which variety *willmorei* was found were up to 2,100 m. at Tapoban in Chamoli District (Bhat, 1975b). Christophers (1933) and Puri (1948) make no distinction between the two varieties in their distributional lists. In the numerous records of *A. maculatus* in Nilgiris, Western Ghats of Old Mysore and North Kanara, etc., no mention is made of the varietal form. According to Christophers the variety is abundant all along the Himalayan region. It is essential to make a distinction hereafter.

There is a record (Puri, 1948) of *A. maculatus* in Thanjavur District. But not a single specimen was collected by the present author and colleagues in that area during their six-years' study.

Adult Bionomics

Resting habits: Observations regarding the occurrence of *A. maculatus* inside houses and cattle sheds have differed considerably. It has been taken quite commonly in houses in many parts of India, particularly Assam and Bengal where it occurs in abundance, but it has also been taken from mixed dwellings in Orissa, Nilgiris, Wynaad, North Kanara, etc. but in small numbers. These are reflected in the numbers of dissections made for malaria parasites. Other observations show that it is collected more in traps than in open houses—suggesting a certain degree of exophily. Being largely a 'feral' species, a marked degree of exophily should be expected.

More studies have been made on this species in other Southeast Asian countries, where it is largely an outdoor rester than in India. The adult enters houses for feeding, but leaves the dwellings for outdoor resting. Adults have been collected in the small vegetation (ferns, etc.) on steep banks in Malaysia and Indonesia and on the forest floor (Wharton, 1950). In Burma, it is not frequent in houses. In Malaysia adults rest outdoors by day in low vegetation and very rarely in houses.

The varietal form, however, seems to be a greater house frequenter than the type form. Shortt (1924) (quoted by Christophers) has definitely commented on the frequency of the species collected in houses during May to August, and also in cattle sheds. Bhat (1975a and b) analysing the data from the Himalayas found that a few specimens were collected indoors in cattle sheds, but mostly they were either collected biting man outdoors or were reared from larvae.

Biting habits: The species bites outdoors as well as indoors. But the exact proportions are not known in India. However, in Malaysia, *A. maculatus* was found to be essentially exophagic (Moorehouse and Wharton 1965) 901 specimens were caught biting outdoors and 289 indoors, approximately a ratio of 3:1 in favour of exophagy. The biting activity in a net-trap commenced soon after dusk and the peak was reached by 22.00 hours. Biting activity fell off markedly after midnight. In biting catches the start of and the peak time of activity were slowed down by two

hours. Both parous and nulliparous components were equally active or inactive at all periods. It also appeared that searches inside houses during the afternoons did not yield the adults of this species.

Moorehouse and Wharton (1965) in Malaysia showed that few individuals bite before 20.00 hours, but about 70 per cent of the total is usually collected in the middle six hours of the night (21.00—03.00 hours) in studies made indoors. Such evidence as there is suggests that this pattern is constant. In Burma, specimens were normally collected in the first and second quarters of the night (18.00—24.00 hours).

Host preference: The records of host preferences are meagre in India. Among the very few specimens collected in Pune District, (Ramachandra Rao and Rajagopalan, 1957), three were found resting indoors, four in outdoor shelters and nine biting men. In Malaysia the species is more attracted to cattle than to man, and bites in large numbers outdoors. When cattle are rare, a high proportion bites man (Wharton, 1951, Davidson and Ganapathy Pillai, 1956).

Horsfall (1972) has given a list of some major series of precipitin tests by various workers. It is seen that the attraction towards man varies from 0.0 per cent in Hong Kong to over 90 per cent in Malaysia. The precentages vary even within the same country, the rate varying according to the place of collection. It appears there is a strain in Malaysia which shows an affinity to man (Wharton, 1950). In the summary of studies on the biting preferences in Malaysia, Reid (1968 Table 14) shows that 41 per cent had bitten man in simultaneous comparative studies between man and calf. Man was more attractive to the species than to a monkey (ratio 14:3). Man-biting rate was quite high (92 per cent) in a man-baited net trap, while with some non-vector species it was very low, e.g. *tessellatus* 15 per cent, *vagus* 3 per cent, *kochi* 16 per cent, etc. (Wharton, 1953). All had similar opportunities to bite man but the actual biting rates varied.

Summarizing the results of precipitin tests done on southeast Asian anophelines Reid (1968 Table 19) has shown that in outdoor shelters, the anthropophilic indices of *A. maculatus* varied from 2 to 14 per cent.

Dispersal and flight: A range of 2.2 kms has been found to be normal in Malaysia. Out of 880 marked individuals, 19 were recovered at that distance, (Galvao, 1948). The varietal form seems to be able to infiltrate into mountainous regions of the Himalayas during warm weather (Gill, 1928, quoted by Christophers).

Colonization: A laboratory colony of *A. maculatus* was established by Yang, Mario and Wharton (1963) by using artificial mating techniques.

Longevity: There are no recent studies, but under laboratory conditions females given one blood meal and subsequently kept alive on sugar solutions had lived upto 66 days (average 34 days). If parous rates can give an indication of longevity, rates ranging from 38 to 53 per cent have been found in Malaysia among a large number examined; they were—134 (38 per cent), 2,031 (39 per cent) and 1,217 (53 per cent) (Reid 1968, Table 20). These could support the view that at least 5 to 10 per cent of the females lived upto two weeks (giving a daily mortality rate of about 15 to 20 per cent estimated by the present author).

Larval Ecology

A. maculatus is primarily a stream breeder, occurring also in ponds, tanks and along edges and in pools formed in the bed of rivers. Christophers (1933) has quoted many references in this regard. This is confirmed by investigations carried out in other Southeast Asian countries, particularly Malaysia. However as is usual with most common species, it is also sometimes found in borrow pits, seepage springs, lake margins, etc. and also in artificial containers. The preference of the species to bright sunlit breeding places is well known and shade has a deleterious effect.

In Burma it has similar habits being found in rocky or sandy pools in beds of hill streams. But the larvae prefer sunlit pools to shaded ones. The presence or absence of vegetation seems to be unimportant. Rarely it is found in rice fields. Breeding seems to be higher in the pre-monsoon and post-monsoon months, the larvae being flushed away during the monsoon almost like *A. fluvialitis* (Khin-Maung-Kyi, 1971).

In Malaysia, it is more prevalent in areas in which trees have been felled and the breeding places exposed to sunlight, at least partially.

The varietal form has similar breeding habits, breeding in the Himalayan hill streams.

Relation to Disease

A. maculatus has often been suspected to play an important role in malaria transmission in India, but rarely incriminated. It can be readily infected with both *vivax* and *falciparum* upto the sporozoite stage (Green and Gater, 1931). It is a very important vector of malaria in some southeast Asian countries, particularly Malaysia.

There have been many dissections in India (See Wattal 1961) commencing from the early days in 1902 by Stephens and Christophers. Except for dissections made by the Assam Medical Research Society reported by Anderson and Viswanathan (1940) for the period 1931—1940 and Viswanathan *et al.* (1941) for periods 1940-1941, no other records of dissections have yielded positive results including 3,374 dissected by Ramsay (1930) in Cachar, and 1,922 by Khan (1942) in Darjeeling and small numbers by workers like Shortt, Strickland, Clyde, Masrat, Ramsay, Iyengar, Senior White and colleagues and Gilroy. In South India, in the Nilgiris, Russell and Jacob dissected 39 with negative results.

The reports of the Assam Medical Research Society were:

	Number dissected	*Positives*		*Area*
		Gut	*Gland*	
1932—41	8,483	24	5	Assam (parts of the area
1941—42	1,573	13	1	are now in Meghalaya)

Therefore one has to be content in stating that atleast in some parts of Assam and Meghalaya *A. maculatus* was a vector.

However, in other countries the records are overwhelmingly clear. In Nepal, the variety *willmorei* has been found infected in nature in Gum Valley at higher altitudes (Pradhan *et al.*, 1970).

In Burma, Hong Kong "Indochina", Kalimantan, Philippines, Malaysia and Indonesia numerous infected specimens have been found in nature. But it is in Malaysia that it has the chief sphere of influence as a vector. Reid (1968) has summarized the more recent studies by various workers and has shown that infections were recorded in Selangor, Southeast Malaysia and Negri Sembelian.

With particular reference to Malaysia, Reid (1968) says that it is the principal vector but is not a vector in many places such as Kalimantan. He attributes it to lesser attraction of man. Harinasuta, Gilles and Sandosam (1976), in their overall review of the vectors in Southeast Asia have regarded *A. maculatus* as a major and important vector in Malaysia, Singapore and of some importance in Kampuchea, Indonesia, Thailand, Vietnam and the Philippines. In Burma, where a few infected specimens have been found in nature, Khin-Maung-Kyi (1971) did not regard *A. maculatus* and its variety *willmorei* as an important vector.

A. maculatus is readily susceptible to *P. cynomolgi* (rather less than *A. kochi*).

A. maculatus is readily infected with *W. bancrofti* but is refractory to the infections with both periodic and subperiodic *B. malayi* and fully refractory to *B. pahangi* (Reid, 1968). It is a vector of *W. bancrofti* in Aur Island of the southeast coast of Malaysia, which has a human filaria rate of 8 per cent. It is probably a vector in other places too.

Anopheles moghulensis Christophers, 1924

Type locality: Quetta, Pakistan, Also specimens from Kasauli (Himachal Pradesh), "Bombay", Satpura Hills, Maharashtra and Madhya Pradesh.

Type: British Museum. Dr. Wattal (Personal communication, 1979) states that the type earlier stated to be in the NICD, Delhi, does not exist there today.

Taxonomy: Originally described by Christophers (1924) as a variety of *A. jeyporiensis.* Before it was differentiated, it used to be identified as *A. jeyporiensis.*

Distinguishing characters: A medium sized mosquito resembling *A. jeyporiensis* and *A. subpictus.* A line of broad overlapping scales in front of wing roots is a specific character. The scaling on the mesonotum is also broader than in *A. jeyporiensis.*

Distribution: India, Pakistan, Afghanistan and Kazakh USSR.

In India: Widely distributed in the north-western, western and Deccan zones. Also in the Punjab and Bihar (Ranchi).

Biology/Bionomics: A rather uncommon species, the adults of which are occasionally collected in houses and cattle sheds. It is known to breed in pools in hill streams and also in seepages of shady banks of streams.

Relation to disease: No information. Too uncommon to be of any significance.

Anopheles pallidus Theobald, 1901

Type locality: Madras and Bihar, India.
(Christophers gives it as Sambalpur).
Type: British Museum.
Taxonomy: There is one synonym, *fowleri* Christophers, 1911 from Amritsar, Punjab.

Distinguishing characters: Adult: Very similar to *A. annularis* but with a brownish complexion rather than black; wings lighter coloured; vein 5 mainly pale except at base and apex; differs from *A. philippinensis* by having scattered pale scales over most of the venter and not merely restricted to the last few apical segments (See key under *A. philippinensis*); and no pale interruption on hind tarsi above white area.

Larva: Differs from *A. annularis* in: Inner clypeal hair with shorter and more chitinized branches; inner sutural hair split into 3-8 branches; differs from *A. philippinensis* in the posterior clypeal hairs with 2-5 branches instead of 7-10 branches; and also in the filaments of the palmate hair being about half as long as blades or more while in *A. philippinensis* it is 1/4 as long as the blades.

Distribution: India, Sri Lanka, Nepal, Bangladesh, Burma, Thailand, Malaysia, Indonesia. Reid (1968) contends that the specimens from Malaysia could really be those of *A. nivipes*. See also Reid, Wattal and Peters (1966).

In India, it is widely distributed in all zones. However, rare or absent in Rajasthan. Not recorded in the Andamans or Lakshadweep.

Prevalence

A. pallidus is an extremely common mosquito, perhaps, more common than *A. annularis*. Generally it is less prevalent in southern most parts of India, but in parts of the Western Ghats it is extremely common and is even the largest component of the anophelines found, e.g. 6,947 out of 8,359 adults (about 85 per cent) in the low rainfall area of Hassan District (Brooke Worth, 1953). In North Kanara District, Karnataka, 6,098 adults were collected out of 42,960, next only to *A. jeyporiensis* (Viswanathan, 1950). Even in Panchmahals District of Gujarat 3,109 adults were collected in a total of 21,422 of all species (about 14 per cent). In the Dhanbad area in Bihar it constituted 4,313 adults out of 33,054 of all species (about 13 per cent) (Sen *et al.* 1960). These figures show that *A. pallidus* forms a sizeable component of the anopheline fauna in many places. It becomes scarcer in the eastern areas. Nair (1973), Jacob (1960), Wattal *et al.* (1967) or Ramachandra Rao *et al.* (1973) found no specimens in high Himalayas, but specimens have been found in the lower Terai regions (Issaris *et al.* 1953).

In Pattukkottai area only 774 adults were collected in a total of 284,591 of all species, a rather low proportion in the population (Russell and Ramachandra Rao, 1941).

Regarding seasons of prevalence, it is a species which thrives in warm weather, but because of its preference to breed in early stages of rice fields, the months of August and September, i.e. the irrigation or the monsoon season, seems to be highly suitable. It does, however, occur all the year round. In dry weather, it breeds in the permanent tanks and ponds.

In Burma, it is an uncommon species. In other southeast Asian countries, it is still scarcer.

Adult Bionomics

A. pallidus is commonly collected in houses and cattle sheds by all workers. While its indoor resting habits are known, its outdoor resting habits are little studied. Presumably a proportion rests outdoors. The present author had collected a few individuals resting on grass. Khin-Maung-Kyi (1971) observes that no adults were collected in Burma indoors but a few were collected outdoors; this may be due to the effect of DDT.

Feeding and biting: Little reliable information exists. In Sri Lanka 13.6 per cent of 119 stomach bloods had human blood (Bruce Chwatt *et al.* 1966). For a species so common, very few observations have been made on this aspect of biology.

Other aspects: Insufficient information. Galvao (1948) quotes Satyanarayana having recovered adults at a distance of 820 metres over open water, presumably in North Andhra coast.

Larval Ecology

A. pallidus is by preference a stagnant water breeder. Christophers (1933) has given references to the breeding in ditches, ponds with vegetation and also in shallow pools in beds of streams. Sen (1941) has shown the relationship of vegetation to *pallidus* breeding. While all aquatic vegetation was favourable to *A. pallidus*, the destructive action of the bladderwort (*Utricularia* spp.) on the species was noted.

In the Pattukkottai area the species was found to have a special predilection to breed in fallow or just planted rice fields disappearing in the later stages. It also occurred in tanks, ponds, field channels. The frequency of occurrence in breeding places in order of preference were (Russell and Ramachandra Rao, 1940):

Rice field growing (early stages)	56.6 per cent
Rice field fallow	39.3
Field channels	27.6
Seepages	18.5
Ditches	16.7
Borrowpits	13.6
Waste irrigation water	12.6

Tanks	11.5
Rain water pools	8.5
Hoof marks, etc.	5.3
Irrigation channels	4.3
Wells	0.8

These bring out the importance of rice fields in the production of *A. pallidus*. It was most commonly associated with *A. culicifacies* and *A. subpictus* and later in the season with *A. nigierrimus* and *A. subpictus*.

It exhibits a marked contrast to *A. annularis* which preferentially breeds in tanks.

Relation to Disease

It has occasionally been found infected with malaria plasmodia in Orissa, east central India (Satpura range) and Bengal at very low infection rates (See Wattal 1961 for detailed list).

The infection rates even in these areas have not exceeded 0.8 per cent as in Birbhum in Bengal found by Iyengar (1939). The largest series of dissections reported is from Birbhum (Bengal) by Timbers (1935) with a sporozoite rate of 0.03 per cent. Sporozoites have been found in Bengal by Sur and Sur (1929) (0.2 per cent).

A. pallidus was found infected in Udaipur State (Roy and Biswas, 1942), a hilly tract in the eastern States agency (150 miles from Chaibasa) in Singhbhum District in Bihar. Out of 854 specimens dissected six were found with sporozoites. At the same time, *A. culicifacies* was also found infected (five gland positives out of 969 dissected). Senior White and colleagues in their extensive in Orissa and east central India, found a few gut positives only.

A. pallidus should be regarded as a malaria vector of some significance locally in Bengal, Bihar and east central India.

A. pallidus can be experimentally infected with *W. bancrofti* but no natural infections appear to have been recorded.

Anopheles philippinensis Ludlow, 1902

Type locality: San Jose, Abra, Luzon, Philippines.
Type: United States National Museum, Washington D.C.
Taxonomy: (See Colless, 1948 for taxonomy).
Formerly confused with *A. annularis* which was then known as *A. fuliginosus*.
Synonyms are:

freerae Banks, 1906 from the Philippines; *pampangensis* Brunetti, 1920 by error, (See Christophers 1933, Colless 1948).

hainanensis Takei, 1941 from Hainan Island, China; till 1968 regarded as a variety (Reid, 1968). *nivipes* (Theobald), 1903 from Malaysia is now regarded as a

separate species by Reid (1967).

None occurs in India.

Distribution: In all countries from India to the Philippines, north upto China (Hainan, Yunnan), but not beyond Borneo and Sumatra in Indonesia.

Not recorded in Sri Lanka.

In India: Mainly in the eastern states of West Bengal, Assam and neighbouring States, Bihar and in scattered localities in Madhya Pradesh, Uttar Pradesh, Orissa, Andhra Pradesh, Karnataka and Maharashtra and in the Andamans. Not found in the western and north western zones or in Lakshadweep.

Distinguishing characters: Adult: A medium sized mosquito, is distinguishable from its two close relatives *A. annularis* and *A. pallidus*, in India by the following key adopted from Christophers (1933).

1. Vein 5 in female with a dark spot in region of origin of branch, and a well marked spot at apex of seg. 1 of hind tarsus: *annularis*
2. Vein 5 in female extensively pale, no interruption, or a not very marked one, at apex of seg. 1 of hind tarsus:

a) No trace of interruption on tarsus; scattered pales scale over most of venter: *pallidus*

b) Varying degrees of interruption on tarsus, rarely entirely without; any scattered pale scales restricted to last few apical segments of venter: *philippinensis*

It should, however, be noted that from the studies in Southeast Asia on the related forms, particularly *nivipes* (Theobald) 1903, there seems to be some overlapping of characters particularly in the wing marking.

Larva: Differs from *A. annularis* by (*a*) the sutural hair being branched and (*b*) the filaments on the palmate hair being shorter and with a mottled pattern distally on the blades.

The points of difference of *A. philippinensis* from *A. pallidus* and *A. nivipes* are given by Reid, Wattal and Peters (1966) in more detail.

It would be of interest to Indian workers in north eastern India to examine their collections of *A. philippinensis* carefully to see if any *A. nivipes* occurs.

Prevalence

A. philippinensis has been found abundantly in Bengal, Assam and neighbouring areas. In other parts of India where it occurs it is generally scarce. All studies prior to 1946-47 in Bengal and Assam had shown that it was very common in the deltaic area, but in some of them there has been a gradual diminution. Today it is reported to have almost disappeared from Bengal. For instance, Arora (1961) could not collect any adults in Durgapur Steel Plant area in Bengal in 1960, and an outbreak of malaria there was attributed to *A. stephensi* or *A. culicifacies*. Neogy and Sen (1962) also, while making a survey of anophelines in rural Bengal, noted the total absence of *A. philippinensis* which was formerly a recognised vector. They remarked that *A. philippinensis* was vanishing gradually from the alluvial deltaic Bengal. The report of N.M.E.P. for 1976 (unpublished—courtesy of the Director,

N.M.E.P.) also shows that *A. philippinensis* had almost disappeared from the State.

Such a disappearance has not, however, taken place in Assam (N.M.E.P. report 1976—unpublished). Rajagopal (1976) collected between 1962 and 1970 large numbers of adults in biting collections in Burnihat (Assam/ Meghalaya) on the road from Gauhati to Shillong in Meghalaya, at an elevation of about 600 metres, *A. philippinensis* is being collected in many other parts of Assam also. However, none or very few adults were collected inside houses and cattle sheds.

The exact cause for the disappearance in Bengal, but not in Assam and neighbouring areas, has not been satisfactorily explained. A suggestion has been made that cytogenetic studies may help in solving the problem. Are there two races? Is the form in Assam a different species? Or has the species changed its habits of resting as a result of DDT? It is worthwhile noting that *A. minimus* has also undergone great reduction in its population, in most parts of the country.

Adult Bionomics

Day-time resting habits: *A. philippinensis* adults in earlier years have been found to rest both in houses and cattle sheds. (Ramsay and Macdonald, 1936), but there are other records to show that they rest mainly in houses (Timbers 1935, Iyengar 1944) or even exclusively in houses (Ganguly, 1947) in the border between Bengal and Orissa. Manson (1951) stated that the proportions resting in houses and cattle sheds in Assam were equal. In Assam were equal. Iyengar(1944) found that 97 per cent were from houses. However, by 1954 Sen (1954) thought that a large proportion rested outdoors as it was difficult to collect them in the houses. In Assam Muirhead Thomson had no difficulty in collecting a few in houses though in small numbers.

In Burnihat (Meghalaya/ Assam), Rajagopal (1976) found that *A. philippinensis* was numerically the most predominant species. It formed 66 per cent of all *Anopheles* collected. While maintaining a high density, it never rested indoors during day-time. Fed mosquitoes were collected at night on vegetation around outdoor shelters. During daytime they went deep into the jungle. This observation of not resting inside houses during daytime is very different from the observations made in earlier years by various authors. The reasons for this difference are not clear. Intense studies both on larvae and adults are to be conducted to satisfactorily explain these differences. However, it would be interesting to note that the outdoor resting habit of *A. philippinensis* is not entirely new. McArthur (1950) had already pointed out that *A. philippinensis* was found resting outdoors in Borneo.

In Burma the species is widely prevalent. Khin-Maung-Kyi (1972) has listed numerous localities in all divisions of the country where this species had been found in recent years from 1962 to 1970. Apparently, the population has not been affected by the DDT programmes, but the adults could not be collected in houses and cattle sheds during daytime in searches made in several districts and only 14 adults were collected resting outdoors. In baited collections, over 3,000 adults were collected on

cattle baits outdoors and only 62 adults on human baits both indoors and outdoors. The search was done in 19 localities over a period of five years. These observations fully support those of Rajagopal in Assam. It is essential to conduct similar experiments in Bengal also.

Biting and feeding habits: There have been wide divergences regarding the observations on biting times. Krishnan (1961) has summarized the observations. The females feed throughout the night, but at different times in different areas. In Bengal:— 20.00 to 22.00 hours and again 02.00 to 04.00 hours; in Malaysia: (Wharton) 21.00 hours to morning; in Burma (Macan):— a small peak before midnight and a main peak between 03.00 and 04.00 hours; or 22.30 to 23.00 hours main peak, 01.00 to 01.30 hours a second peak.

Khin-Maung-Kyi (1971) however, pointed out that in Burma intense biting activity occurred during the first quarter of the night (18.00-21.00) hours as revealed by catches on both man and cattle, forming 60 to 80 per cent of all *A. philippinensis* collected during the night. Rajagopal (*loc. cit.*) in Burnihat found that *A. philippinensis* was found to bite man all through the night starting from 18.30 hours to 04.00 hours with peak activity between 19.00 and 22.00 hours and again from 01.00 to 04.00 hours. (He found only seven adults resting inside houses over a period of two months).

A. philippinensis feeds both on man and cattle. There are few records of precipitin tests of stomach bloods, but taking the numbers actually found biting man and cattle some significant conclusions could be drawn. In Assam Ramsay and Macdonald (1936) found 6.3 per cent of 343 stomach bloods positive for human blood. In Malaysia, Hodgkin (1945) had found 42 (37 per cent) of 113 stomach bloods positive for human blood. These collections were made in a man-baited trap near houses at night, but with cattle sheds not far away. Summarizing the results of precipitin tests of stomach bloods from mosquitoes caught resting outdoors by day in several parts of southeast Asia, Reid (1968—Table 19) has shown that out of 699 positive stomach bloods from *A. philippinensis* only 10 had human blood and 689 cattle blood.

Rajagopal (*loc. cit.*) in Burnihat found that in all night collections,1,417 *A. philippinensis* feeds both on man and cattle and 187 biting man. In fact *A. philippinensis* formed the bulk of all 12 species of *Anopheles* collected there (total on cattle 2,130 and on man 282). Wharton's (1951) observations in Malaysia showed that in window-trap huts with man and calf, 567 were collected on calf and 10 on one or two men. Similar results have been reported by Reid (1961) in Malaysia and Eyles *et al.* (1964) in Kampuchea. The percentage biting man was only 6 per cent. These figures, however, are comparable.

Therefore, *A. philippinensis* is predominantly a zoophilic species but quite good numbers also bite man and its anthropophilic index is much higher than that of *A. culicifacies.*

Flight and dispersal: Very few critical observations have been made on the flight and dispersal of the species. Sen (1954) thought it was a moderate flier not able to fly beyond 0.4 to 0.8 km.

Larval Ecology

A. philippinensis is known to breed in a variety of breeding places, particularly swamps, tanks, ponds, ditches, rice fields, etc. generally with good growths of vegetation and with stagnant water.

It has long been well known that *A. philippinensis* shows a preference to breeding in tanks and ponds (Covell, 1927), a habit which it has in common with its close relatives, viz. *A. annularis,* and *A. jamesii.* Bose (1931), Ramsay and Macdonald (1936), Roy and Roy (1941) and Iyengar (1942) have all shown the relationship of presence of aquatic vegetation and the breeding of the species. While all types of aquatic vegetation are favourable, it appears that certain plants such as *Lemna* (duck-weed) and *Eichhornia* (water hyacinth) are inimical to this species, Iyengar (1942-44) thought it was mainly due to their effect on the growth of plankton, such as blue green algae, which seem to be the cause of decline in the breeding. Rice fields as sources of this species have been intensely studied. Sen (1933, 1941 and 1948) as well as Senior White (1946) have given considerable attention to this subject. Sen's findings may be summarized as follows:—

"*A. philippinensis* was comparatively uncommon in rice fields never forming more than one per cent of the total collections of larvae" and appeared relatively later in the season. "The breeding again declined with the last stages of the rice growth because the water at the base of the plant began to accumulate rotting vegetation. The species was not found in fallow fields with turbid water and appeared only when the plants were 12 to 18 inches (30 to 45 cms) in height and the water was no longer turbid."

According to Sen (1941) the relative frequency of *A. philippinensis* breeding in ponds overgrown with aquatic weeds was usually 35 times as large as in rice fields.

Quraishi *et al.* (1951) during their study of the pre-monsoon transmission of malaria in Bangladesh (formerly East Bengal of Pakistan), made some interesting studies of the breeding habits of *A. philippinensis.* They found that there was a positive connection between malaria incidence and proximity of villages to marshy land or dead rivers. Owing to the fact that in the plains of Bangladesh the river system is extensive, rivers often change their courses and the number of dead rivers is considerable. These marsh lands and dead rivers were ideal breeding places for the species. In fact there was greater abundance of *A. philippinensis* adults in villages near the marshy lands and dead rivers than in villages near typical and usual breeding places such as tanks and ponds.

In other southeast Asian countries, McArthur (1950a) found that *A. philippinensis* was typically breeding in rice fields as also in irrigation channels and in waters generally with grassy edges where the water was gently flowing. His observations were similar to those of Sen (*loc. cit.*) in that, breeding increases as the paddy plants grow and the breeding declines completely when the flow of water was cut off and stagnation commences. In Burma, Khin-Maung-Kyi (1972) states that the species is regarded as a stagnant water breeder and found in rice fields, tanks, borrow pits, swamps, ponds with grassy edges or emergent vegetation. They were frequently found in water collections covered with dense growth of algae. It is often found

breeding in association with *A. annularis, A. "hyrcanus"* and *A. aconitus.*

Iyengar's (1944) observations on the aquatic ecology of the species deserves further comments. The aquatic vegetation of the submerged type which reached the water surface was favourable for the breeding of the species. The aquatic plant most frequently found associated was *Hydrilla.* However, aquatic plants which emerged over the water surface producing shade were inhibitory. The dense growth of the water hyacinth controlled the breeding of this species effectively. The effect of shade seemed to be an indirect one affecting algal growths, particularly of green algae which provided the main food for the larvae and favoured the development of the larvae. Certain other algae, such as *Microcystis aeruginosa* and *Euglena* sp. were unfavourable.

Sen (1941) has also recorded that larvae of *A. philippinensis* were found in Bengal in water containing all types of plants studied, except *Lemna,* but the species was most frequently associated with the filamentous alga, *Spirogyra.*

A very interesting finding of Iyengar (1942) was that the highest malaria prevalence in Bengal was at the time when the water table was low. This was the time when the breeding of *A. philippinensis* was also at its peak. Raising of the water table seemed to affect the breeding of this species. He even postulated that if there is intensive raising the water table in deltaic Bengal the incidence of malaria could be controlled. The exact mechanism of this phenomenon is not understood. (It should, however, be noted that the species is reported to breed intensely between July to October, the monsoon period when the area gets flooded. These are somewhat contradictory).

Relation to Disease

In 1933, Christophers said that there was no evidence to suggest the relationship of *A. philippinensis* to disease, a very surprising statement in view of the subsequent recognition of the species as an important malaria vector. Even in 1928 and 1929 Sur (1928) and Sur and Sur (1929) had found natural infections in Bengal; three gut and 18 gland infections in 993 females dissected. But, extensive series of dissections made earlier and subsequently by workers such as Strickland (1929), Ramsay (1931), Ramsay and Macdonald (1936) and others with well over 22,500 dissections, no positive had been found. Iyengar, however, showed the species to be a vector in Bengal after the early records of Sur and Sur.

This disparity between the earlier and later records is intriguing and inexplicable now. Ganguly (1947) and Sen (1948) confirmed Iyengar's observations. Some results of important positive dissections were:

		Total number dissected	*Positives*	
			Gut	*Gland*
Iyengar	1939	1,928	81	76
	1940	3,788	157	141
	1944	3,165	149	126
Ganguly	1947	294	5	—
Sen	1948*a*	1,133	Total 101 infections	

In Assam, Anderson and Viswanathan (1941) recorded two gut and two gland infections, in 4,239 dissections between 1932 and 1940. Rajagopal (1976) found one gland positive out of 195 dissected in Burnihat. But in east central India and Orissa Senior White and colleagues have recorded no infections.

In West Bengal State, *A. philippinensis* is one among the three important malaria vectors. Iyengar as well as Sen have described the vectors of Bengal as follows:

Foothills of Himalayas	... *A. minimus*
Deltaic Bengal	... *A. philippinensis*
Estuarine areas	... *A. sundaicus*

In Bangladesh, Quraishi *et al.* (1951) found three sporozoite infections in *A. philippinensis* out of 1,271 dissected; one in May and two in June. They emphasized the observation that there was much pre-monsoon transmission, in addition to the post-monsoon transmission previously recorded by Sinha (1942) and Iyengar (1944).

In Burma, it is a local vector of minor importance in the Burma-Bangladesh border.

As Reid (1968) has pointed out this species, like *A. maculatus,* is very variable in its geographical range. It was among the poorest vectors in the order of importance which he listed for southeast Asia.

A. philippinensis is not recorded as a vector filariasis in India though experimental infections with the *bancrofti* have been obtained (Raghavan, 1969, Das, 1976). There is no evidence to suggest that it has any role in transmission of human filariasis in any part of southeast Asia.

Anopheles pulcherrimus Theobald, 1902

Type locality: Lahore, Pakistan.

Type: British Museum.

Taxonomy: One synonym; *atropotenae* Lindtrop, 1924 from Azerbaijan (USSR).

Distribution: India, westwards through Pakistan, to Afghanistan, USSR (Khazak, Caucasus) Iran, Iraq, Saudi Arabia, Behrain, Syria, Lebanon, Israel, Turkey. An essentially West Asian species.

In India: In the north-western regions in the Punjab, western UP and in Gujarat.

Distinguishing characters: Adult—a large sized mosquito. Because of the presence on abdomen of dense scales with projecting lateral tufts, it has a shaggy appearance. From its close relatives of the *Neocellia* group, it can be distinguished by having the hind legs unspeckled, the distal part of tarsi with 3¾ segments continuously white and the above mentioned lateral tufts of scales on the abdomen.

Larvae—Outer clypeal not brush like, but with only 4-12 branches; palmate hair well developed on metathorax and abdominal segments I-VII.

Prevalence

A species of the semi-arid regions of West Asia. In India also confined to the northern and north western regions, apparently the eastern limits of its range.

Adult Bionomics

Resting habits: Rests in houses and cattle sheds. In the main region of its distribution, as for instance in northern parts of Afghanistan bordering the USSR, it occurs in enormous numbers in houses and cattle sheds. Some outdoor resting does take place (personal observations of the author). So also in Sind and Pakistan Punjab. According to Covell (1944) the adults prefer to rather rest in partially shaded places in houses than in deep shade. In the Changa-Manga Forest near Lahore, it was the third most common mosquito. (Aslamkhan and Salman, 1969).

Biting habits: It bites man readily and also on cattle. There is reluctance to bite indoors or to enter animal baited traps (Ludlam quoted by Reisen and Aslamkhan, 1978). The latter authors organized all-night biting collections on man in a village near Lahore and collected 2,789 specimens during a one year period. It was the most abundant anopheline followed by *A. annularis* (2,568) and *A. culicifacies* (1,736). Though small numbers were collected at all hours of the night, the species typically fed before midnight. There were some seasonal shifts, the biting extending a little into the third and fourth parts of night in the warmer months. In Changa-Manga Forest 12 adults were collected during daytime supporting the earlier observations reported by Christophers (1933).

Flight and dispersal: It is quite a hardy species. Wright (1917) found a swarm of this species over a ship about 25 kms from land in Shat-el Arab in the Persian Gulf. The adults should have either flown or were drifted in air currents upto that distance.

Host preferences: In a comparative study in 1964, Aslamkhan and Salman (1969) found only 120 adults biting man, but 3,247 biting a cow during biting collections made outdoors between 18.00 and 21.00 hours. In Afghanistan, according to the personal experience of the author, many more adults were collected in stables, than in pure human dwellings.

Larval Ecology

Breeding places of *A. pulcherrimus* are ground pools like borrowpits with good growths of vegetation. Rice fields are very good sources in Pakistan and Afghanistan. In USSR, shallow lakes are favourable breeding places. Very little information on breeding habits in India is known.

Relation to Disease

The few dissections, totalling about 153, made in India and Pakistan have all been negative. However, Onori (1975) considered that *A. pulcherrimus* was a vector in northern Afghanistan. Covell (1944) also states that the species is a vector in southern parts of USSR.

Anopheles ramsayi Covell, 1927

Type locality: Cachar, Assam.
Type: British Museum.

Taxonomy: One synonym, *pseudojamesii* Strickland and Chowdhury, 1931 from Bengal.

Distribution: India, Nepal, Bangladesh, Burma, Thailand, Malaysia, Indonesia, Sri Lanka.

In India: Restricted to north eastern parts only (Assam, West Bengal, Orissa and Bihar).

Distinguishing characters: Adult: a medium sized mosquito. Easily distinguished from its closest relative, *A. jamesii,* by absence of golden coloured scales and hairs on the apical segments of the abdomen and by darker wing.

Larva: Outer clypeal hair half to 2/3 of the inner, short with 3-8 short lateral branches and not so highly branched as in *A. jamesii.*

Bionomics/Ecology: Adults found in houses and cattle sheds in small numbers; breeding places are rain water pools, tanks and swamps with heavy growths of vegetation (Covell, 1944).

Relation to disease: Stray specimens have been found infected in Assam, Bengal and Orissa, mostly oocysts and only two sporozoite positives, in several thousands dissected.

At most a poor and insignificant vector.

Anopheles splendidus Koidzumi, 1920

Type locality: Taiwan.

Type: Location unknown.

Taxonomy: One synonym, *indiensis* Theobald, 1903 from Nagpur, Maharashtra. As the name *indiensis* is already preoccupied the name of *splendidus* to this species is correct. The name *indiensis* has also now become non-existent. (See under *A. nitidus*).

Another synonym viz. *maculipalpis* (James and Liston, 1904) formerly regarded as a synonym of *indiensis* is not recognized, as the name of *maculipalpis* is also preoccupied for a species which occurs in Africa and hence is not valid. (*A. maculipalpis* Giles, 1902).

Distinguishing characters: Distinguished from its close relatives such as *A. jamesii* and *A. ramsayi* by the following combination of characters:

Female palpi with two broad apical pale bands, and with conspicuous speckling; male palpi with shaft banded and spotted with white; black and white scales on last segment of abdomen and not golden scales or hairs.

Distribution: India, Pakistan, Afghanistan, Nepal, Bangladesh, Burma, Thailand, 'Indochina', China, Taiwan.

In India: In most zones of India: Punjab, Kashmir, Himachal Pradesh, West Bengal, Sikkim, Assam, Madhya Pradesh, Maharashtra, Gujarat, Karnataka, Andhra Pradesh, Kerala, Tamil Nadu and Goa.

Ecology/Bionomics: Generally a species of hilly terrain but fairly common in several localities.

Except for its wide prevalence, very little is known about its biology. It is found

in small numbers in houses and cattle sheds. Primarily a cattle feeder but may occasionally bite man.

Larvae have been found breeding in beds in hill streams.

Relation to disease: No evidence that it plays any part in malaria transmission. In a moderate series of dissections in India, only once a gut infection was found in the Punjab, but infections have been found in Hong Kong and Taiwan.

Anopheles stephensi Liston, 1901 and variety *mysorensis* Sweet and Rao, 1937

Type locality: Ellichpur, Vidarbha area of Maharashtra State, India.

Type: Location unknown. The original description is that of the female.

Taxonomy: There are three synonyms and one variety— *metaboles* Theobald, 1902.

Type locality: Lahore, Pakistan, *intermedia* Rothwell, 1918.

Type locality: Deesa, Gujarat State. *folquei* de Mello, 1918.

Type locality: Nagarhaveli (Pragana) (formerly a Portugese Colony) now part of Dadra and Nagar Haveli in the Gujarat region.

Var. *mysorensis* Sweet and Rao, 1937.

Type locality: Unknown but in Karnataka State.

Type: Location unknown.

Puri (1949) raised *mysorensis* to the sub-species status, but Knight and Stone (1977) have listed it as a synonym. It is being treated as a variety in this publication because there are adequate grounds to differentiate it on behavioural features if not on genetic basis.

Distribution: India, Pakistan, Afghanistan, Iraq, Iran, Bahrain, Oman, Saudi Arabia, Bangladesh, South China, Burma, Thailand. Not reported from Nepal and Sri Lanka. The species has been found in Egypt, 1.5 kms from the Suez Canal, the first record on the African continent (Gad 1967).

In India, it occurs in all mainland zones becoming rather scarce at high altitudes in the Himalayas. Not found in the Andamans or Lakshadweep.

Distinguishing characters: The adult is a medium sized mosquito and can be distinguished from all the closely related species, indeed from all other Indian anophelines, by having (*a*) two broad apical pale bands on the speckled palpi; (*b*) broad scaling on thorax; (*c*) hind legs not conspicuously marked in white; and (*d*) legs speckled.

The larva can be distinguished from close relatives by (*a*) the absence of palmate hairs on thorax; (*b*) filaments of abdominal palmate hair half or less than half of the length of the blade; (*c*) outer clypeal always simple and inner clypeal usually so.

The type form and the variety *mysorensis* can be distinguished between themselves only by egg characters (Rao, *et al.* 1936). The averages of 4,707 eggs from 105 *type* females measured by them were:

Length:	555 microns	SD	± 24	microns
Breadth:	204 microns	SD	± 12	"

Length of float: 294 microns SD ± 23 microns
No. of ridges on float 18 SD ± 1.6
In 6,916 eggs from 147 *mysorensis* females, the measurements were:
Length: 476 microns SD ± 24 Microns
Breadth: 106 microns SD ± 12 "
Length of float: 218 microns SD ± 20 "
No. of ridges on float 13 SD± 1.2

They also measured the length and breadth of the wings and it was concluded that they were not of practical use to detect the two forms.

No other morphological differences have been noticed. It is yet to be seen whether cytotaxonomy would give any clues.

Bhatnagar *et al.* (1958) have found specimens of *A. stephensi* with the dark colouration missing (hypomelanic form).

Prevalence

The type form is predominantly an urban mosquito while *mysorensis* is largely rural in distribution (Viswanathan, 1950). There are no clear seasons of prevalence in most parts of the country so far as type form is concerned, but *mysorensis* has seasonal variations similar to those generally associated with species like *A. culicifacies* and *A. fluviatilis*. But in southern India, Russell and Ramachandra Rao (1941) found more numbers in April and May than in other months though facilities for breeding were present throughout the year. Recently Rajagopalan *et al.* (1979) have found that in Pondicherry the well-breeding *stephensi* occurs in most months of the year except during the monsoon.

In Bengal, Chaudhury (1936) found that *A. stephensi* was more prevalent in seasons with average rainfall not less than 5 cms and relative humidity of about 85 per cent and temperatures between 77° and 89° F.

Both the type form and the variety are present in all the mainland zones of the country. Outside India, it had been presumed that the varietal form did not occur beyond the Indo-Pakistan region, but it is now known that *mysorensis* occurs widely in Iran also and is a malaria vector of importance.

According to Sweet and Rao (*loc. cit.*) the two forms were found at different places such as:

Type (B)	*mysorensis (M)*
Bangalore City	Mysore State (Rural)
Mysore City	Pune
Delhi	Calcutta
Pune	Sukkar (Sind)
Calcutta	Hyderabad (Sind)

Viswanathan (1950) is on record that *A. stephensi* of the Deccan plateau were all of the form *mysorensis*, but in Pune City both the forms occurred (unpublished data). Senior White (1940) found that in Calcutta also both the forms were present. In the Nira Canal Zone of the Deccan, it once formed nearly a quarter of all the

adults collected, but in 1979 it was difficult to collect even a dozen specimens. There undoubtedly has been a change.

Adult Bionomics

Resting places: Adults have been collected from houses, cattle sheds, barracks, etc. However, it is generally regarded that in eastern India, particularly in Calcutta, adults are not easily collected in houses. Senior White (1940) summed up the information by stating that the daytime resting places of the species in Calcutta were obscure. Ramsay and Macdonald (1936) had also remarked on this difference in prevalence in eastern and western India. In Bombay, it was a house-haunting mosquito, while in Calcutta it was not so. Knowles and Basu (1934), Ganguly (1935) and Strickland *et al.* (1936) have brought out the scarcity of the adults in the eastern region. In Salem Town recently (Reuben personal information 1979) has been finding it difficult to collect enough numbers of adults in human and animal dwellings commensurate with the degree of breeding which occurred in wells.

However, adults are not rare in all places. In the Deccan, where *mysorensis* predominates in indoor collections, the species was very abundant (Viswanathan, 1950).

	Numbers collected	
	stephensi	All *anophelines*
Dharwar & Bijapur Districts	405	5,885
Poona—Sholapur	8,373	52,790
Nira Canal Zone	57,279	191,236
Nasik	1,820	70,719
West Khandesh	5,673	121,198
East Khandesh	1,565	49,929

As a matter of fact, it was the most abundant species in the Nira Canal Zone, followed by *A. culicifacies* and *A. fluviatilis*. Perhaps these differences in resting habits could be due to the fact that in the Deccan rural areas *mysorensis* is prevalent, while in Calcutta and Salem the type form is more prevalent.

In another part of the Deccan (Bellary in Karnataka State), Bhaskar Rao, *et al.* (1946) also collected large numbers inside houses and cattle sheds, 18 per cent in human dwellings, 32 per cent in mixed dwellings and 32 per cent in cattle sheds.

In Pakistan and Iran, there have been several studies on *A. stephensi*. Mulligan and Baily (1936) in Quetta found that out of 1,200 adults collected, 1,119 were collected in or near houses. Afridi and Majid (1938) working in Bahrain in the Persian Gulf, found them resting inside houses close to breeding place. Reisen, Aslamkhan and Naqvi (1976*b*) found in Lahore District of Pakistan that adults were resting in cattle sheds and none was collected out of doors, while *A. annularis* was found to be both endophilic and exophilic.

Records of outdoor resting are meagre, and perhaps like most other species a certain proportion does rest outdoors. In Iran, however, Quraishi (1965) captured 2,083 adults in a pit shelter, strongly suggesting that *A. stephensi mysorensis* has a

strong outdoor resting component, at least in that country.

Regarding the actual surface on which the adults rest, the present author has not found any difference between *A. culicifacies* and this species either in Tamil Nadu or in the Deccan plateau. Other observations, like those of Bhaskar Rao, *et al.* (1946) in Karnataka and Rafi (1955) in Pakistan Punjab, have also shown similar resting habits, that is, the adults being found in houses at all heights.

A. stephensi adults are influenced by temperature and humidity in the selection of the resting places. Dakshinamurthy and Sharma (1951) carried out laboratory experiments in chambers with humidity and temperature gradients and found that at all temperatures above 30°C, *A. stephensi* and *Culex fatigans* did not exercise any choice at all. They concluded that the choice of humidity depended on temperature and the adults selected places in which there would be least loss of moisture. In temperature studies, *A. stephensi* exerted a choice at 25°C when the choice was between 20°—25°C and 25°—30°C.

Biting and feeding habits: Early observations of Chaudhury (1936) indicated that adults were most active in biting before mid-night in Calcutta. In Iraq, (Ramakrishnan quoted by Krishnan 1961), the biting was over before mid-night. In the former Mysore State, Nursing *et al.* (1934) collected 33 per cent with fresh blood between 21.00 to 24.00 hours and 67 per cent between 04.00 and 06.00 hours. Whether these represent the actual differences in behaviour or are merely due to differences in technique is not clear.

In Pakistan, Reisen and Aslamkhan (1978) made the following observations: "*A. stephensi* feed mostly before midnight being markedly crepuscular during periods of low ambient temperature." They made a critical study of the differences between seasons, *i.e.* February, April, May, July, October, November and December and concluded (agreeing with Eshghy and Janbaksh, 1977, in Iran) that during warmer months of June to November biting rates on both man and cattle were highest during the second quarter of the night. In January and February, there was practically no biting after 19.00 hours and the biting was actually crepuscular, but in November and December some biting took place also in late night. They stated that they had made similar observations in Karachi also. These studies which are the most critical and purposeful ones on biting rhythms made hitherto have brought out the importance of seasons in regulating the biting activities. How much of the differences noticed by different authors are due to this fact alone needs further study.

Flight and dispersal: Very little precise information, except for one study referred to below, exists on flight and dispersal. But on indirect information, the species is regarded as a moderate flier upto half a mile (Covell, 1944). In Port Sandeman in Pakistan, De Burca and Jacob (1947) found the species in houses about 2½ miles away from the closest breeding places. Similarly Afridi and Majid (1938) had collected adults in houses 1½ miles from the nearest breeding places. Galvao (1948) has dealt with the matter, and from a review of literature stated that adults of this species had a normal flight range of 0.8 to 2.5 kms. Covell (*loc. cit.*) had also referred to flights upto 2.4 kms over continuous stretches of water. Like many other

anophelines, this species can also be transported over long distances by train (Sen 1941).

In an experiment in Iran, flight characteristics of *A. stephensi mysorensis* have been studied by Quraishi *et al.* 1966. Using P^{32} tagged mosquitoes they released over 190,000 adults including both males and females in four batches in two locations in July and August 1962. Subsequent collections were made in 15 experimental villages. Searches were made in bed-rooms, stables, magoon traps and pit shelters. They recaptured, over a period of nine days, 51 males and 132 females which were radio-active. No specimens could be found after nine days. The numbers recaptured at several distances from one release point namely Sha-us-Saltaneh, were as follows:

Distance	*Males*	*Females*
0.2 kms	42	115
1.0 kms	2	4
1.5 kms	0	1
1.8 kms	1	2
2.0 kms	0	2
2.8 kms	1	2
3.4 kms	3	4
3.7 kms	1	1
4.5 kms	1	1
Total	**51**	**132**

The number collected on different days were:

Days after release	*Males*	*Females*
1	18	54
2	12	46
3	6	25
4	3	15
5	4	11
6	4	6
7	2	2
8	0	3
9	0	1
Total:	**49**	**163***

*These totals include recaptures made from another release point 30 kms away and therefore do not tally with the figures given above.

They grouped the villages into two zones: one at 1.3 kms and another at 3.5 to 5

kms. They compared the proportions of marked mosquitoes to the total catch in each zone with the following results:

	Males	*Females*
1.5 kms	0.0665 per cent	0.0481 per cent
3.5 kms to 5 kms	0.0124 per cent	0.0082 per cent

These figures indicated a marked fall in the tagged mosquitoes from 1.5 to 3.5 kms. In a previous preliminary study they had felt that *A. stephensi mysorensis* had a much longer flight range than 2 kms and the present study supported their observation.

In view of the small numbers recaptured they did not feel justified in drawing any broad conclusions except to state that the date showed a rough trend, i.e.recaptures could be made at distances upto 4.5 kms and for 9 days after release. But the males and females were found to fly 1.8 kms overnight. From an analysis of the recaptures it was clear that the vast majority (42 males and 115 females stayed with in a kilometre).

Host preference: Though *A.stephensi* is an important malaria vector, it bites cattle predominantly. Precipitin tests have been carried out by many workers and some or the anthropophilic indices recorded were: Afridi *et al.* (1939) Delhi 1.4 per cent; Roy *et al.* (1938) Bengal 13.4 per cent; Bhaskar Rao *et al.* (1946) Karnataka Deccan 0.8 per cent. In all these areas, the stomachs contained cattle blood predominantly.

Nair and Samnotra (1964), however, found in Broach town in Gujrat, a human index of 37.5 per cent out of 49 fully fed *A. stephensi* collected from mixed dwellings, quite a high rate, but not to be unexpected because of the comparative lack of cattle in the urban area and the intense malaria transmission going on in that town at that time (1964). The form in Broach Town was in all probability the type-form.

In Salem town, Tamil Nadu, Reuben and colleagues have found in night collections, 44 specimens of *A.stephensi* biting man while 65 were collected biting cattle. It is interesting to note that during the same collections, only 133 *A. subpictus* were collected on man while 12,010 were collected biting cattle indicating that *A. stephensi* was definitely better attracted by man than was *A. subpictus* (Batra *et al.* 1979).

Swarming, mating and colonization: There are no reports of swarming and mating having been observed in nature in India, though very good observations have been made in laboratory colonies.

In Iran, however, swarming and mating have been observed in nature (Quraishi 1965). *A. stephensi mysorensis* was observed swarming in village Shau-Us-Saltaneh, Kazerum. Swarming commenced after 18.30 hours and occurred above small tres and roof-tops. Several small swarms gathered together to form larger swarms. About 50,000 males were estimated to be in the swarms each evening. The females entered the swarms and several males attacked them and the mating pair fell to the ground. An average of nine females entered the swarm each minute in a swarm consisting of 500-600 males. The females which entered the swarms included nulliparous, uniparous and biparous individuals all with empty stomachs.

A.stephensi is one of the species amenable to very easy laboratory colonization. Rao *et al.* (1938) established successfully a colony of the species. While they were successful with the type-form, they did not succeed with the *mysorensis* form. Subsequently Russell and Mohan (1939d) established very good colonies in small cages of the size 60 x 60 x 60 cms. Among many observations they made on the behaviour of the adults, a few important ones were:

a) a swarming type of activity occurred at about 18.30 hours during which mating was noticed;
b) a previous blood meal was not essential for successful mating;
c) pairing occurred both with nulliparous and multiparous females;
d) pairing was not necessarily dependent on any stage of ovarian development;
e) *A. stephensi* males attempted to mate even with females of other species, such as *A. annularis, A. subpictus* and *A. culicifacies;*
f) transfer of sperms was also achieved if copulation took place during flight.

Colonies of *A. stephensi,* including mass rearing, have been maintained in many laboratories in the world by quite simple techniques.

In Delhi, Ansari *et al.* (1978) have also developed newer methods of mass rearing of *A. stephensi.*They found that a cycling colony was better than a non-cycling colony for obtaining maximum egg production per female. They used standard wooden cages (70 x 60 x 60 cms). Adults were fed on water-soaked raisins and were offered blood meal on rabbits. Eggs were collected in ovipositional jars (6 x 8 x 10 cms) containing 250 ml.of water and lined with filter paper. Eggs were held in these jars for 72 hours for the hatch. Newly hatched larvae were transferred to plastic rearing trays measuring 63 x 60 x 9 cms. Larvae were fed on powdered food which consisted of 60 per cent dog biscuit and 40 per cent brewer's yeast. Pupae were collected from the trays and they were separated from larvae by chilled water. They obtained an average number of eggs ranging from 42,713 to 72,000 per day. The average number of eggs per female was 2.5 to 3.4 in the non-cycling colony and from 30.6 to 63.5 in the cycling colony.

Initially there were some doubts whether the form *mysorensis* could be colonized. Rao *et. al.* (1938) were unsuccessful, but later, success has been obtained by several others in India, Pakistan and Iran.

Light preferences: In laboratory studies *A. stephensi* females have been found to show some important preferences for certain colours (Wilton and Fay, 1971). Using monochromatic light they found strong positive reactions to the middle ultra violet wave length green portion of the spectrum were comparatively unattractive. Recently Reuben *et al.* (1979) have found in their field studies in Salem town that the females of *A. stephensi* at night were easily disturbed by the yellowish light thrown by flash lights. But when the flash lights were covered by a red cellophane paper, the adults were not disturbed at all. By using this technique they have reported collecting better numbers of the females.

Longevity: During studies on experimental infections, Knowles and Basu (1934) made a few observation which will be referred to later. Observations on caged mosquitoes have shown that at relative humidities of above 55 per cent individual

specimens could live upto 32 days (Mayne, 1936).

Strickland and Roy (1936) had specimens living upto 36 days. *A. stephensi* is reported to be a hardier mosquito than *A. culicifacies* because it could live a little longer under dry conditions. In Iran Quraishi *et al.* (1966), during a study on flight of *A. stephensi mysorensis* estimated that mortality appeared to be about 30 per cent per day. Using Detinova's methods they graded 322 females of which 299 were nulliparous, 62 uniparous and 17 biparous.

In Broach Town, Gujarat, Nair and Samnotra (1964) showed that out of 61 females dissected only nine were nulliparous, 22 uniparous, 192-parous and 11 3-parous, showing that at least 19 per cent were living upto the epidemiologically dangerous age.

There is no indication that gonotrophic cycles of *A. stephensi* are any different from those of other common species like *A. culicifacies* or *A. annularis*. However, Strickland and Roy (1936) made an unusual observation that at summer temperatures, the cycle took 4 to 11 days to mature a batch of eggs. Under laboratory conditions the gonotrophic cycle took 2 to 3 days, the first cycle perhaps taking a day more (Russell & Mohan, 1939).

Quraishi *et al.* (*loc. cit.*) also made some observations on the longevity and gonotrophic cycle. They found the daily mortality rate was exponential. They used ratios of radioactive tagged specimens to the total captured on any one day, using data only for females. The data when plotted, on a semilog paper, showed a straight descending line. The first gonotrophic cycle was completed in 3 to 4 days and the second in 6 to 8 days after eclosion.

Larval Ecology

A. stephensi larvae are found in many types of breeding places. In urban areas they are predominantly found in man-made breeding places such as wells, overhead or ground level cistern, roof gutters in factories, barrels, buckets, ornamental tanks, etc. There are many references to early observations in this regard and have been quoted by Christophers (1933) and Krishnan (1961). The breeding of *A. stephensi* in wells, cisterns, fountains, etc. in Bombay City even in 1904 has been described by Bentley (1911). A recent reference breeding in man-made breeding places is that by Dhir (1969) in Delhi City when a sharp rise in malaria incidence was investigated and it was found that *A.stephensi* larvae were breeding in water collections in buildings under construction. Water was flooded over newly laid concrete floors for purposes of curing cement. Breeding of *A. stephensi* such situations led to the epidemic. Covell (1928) studying malaria in Bombay City has stressed the importance of man-made breeding places.

In rural areas the species, mainly the *mysorensis* variety, has a much wider range of breeding habits. It breeds in streams and channels, tanks and ponds, seepages, irrigation wells, etc. Such a wide habit has been known for a long time. Among studies made in the Deccan Plateau (reported by Viswanathan 1950), in Bijapur District, the breeding places of *A. stephensi* were rivers and streams followed by

borrow pits. In the Nira Canal area of Pune and Sholapur Districts, where the species was the most abundant of all anophelines, the present author found *A stephensi* breeding intensely in a large number of seepage-filled *nullahs* (streams), overgrown with bull-rush, in association with *A. fluviatilis* and *A. culicifacies*. That *A. stephensi* does not however, breed in natural breeding places in all areas is seen from the observations of Russell and Ramachandra Rao (1940) in Pattukkottai area. In an intensive two-year-study of 6,033 breeding places, *A. stephensi* larvae were found only in domestic wells (814 larvae in 37 wells out of a total 1,240 wells searched). *A. stephensi* larvae were not found in any of the other types of places.

However, the habit of breeding in natural breeding places has been recorded from a number of other countries such as Afghanistan, Iran, Pakistan and also many parts of India both in the west and the east. Even in Burma, "In the foothill areas from which the species have been mainly recorded the larvae been found in pools in river beds, in seepages and along edges of rocky hill streams". (Khin-Maung-Kyi, 1971). There is strong reason to believe that the different types of habits are due to the existence of two biological forms of the species (see below for discussion of the studies of the two forms).

While the species generally prefers clean waters, it is not averse to breeding in highly polluted water. Roy (1931) had shown that in Calcutta it was breeding in waters actually contaminated by sewage. Russell and Mohan (1939), in a study of the effect of the chemical composition of water on susceptibility of the species to plasmodial infections had actually reared larvae in basins containing water with sullage and having an ammoniacal nitrogen content of 55 per cent. A hypothesis was once being advanced that the ability of *Anopheles* species to transmit malaria could be influenced by the chemical contents of the water they grew up. It was postulated that adults emerging from larvae breeding in highly nitrogenous waters were likely to be poor vectors. It was to test this hypothesis that Russell and Mohan (1939) reared *A. stephensi* larvae in many types of waters including one contaminated by sullage water and found no difference in the ability of the adults to transmit malaria.

The larvae can also breed in brackish or salt water (Chalam, 1926). In Bahrain in the Persian Gulf *A. stephensi* was found breeding in slightly saline water in pits with salinity ranging from 0.13 to 0.27 per cent (Afridi and Majid, 1938). In Bombay City, Bana (1943) found that the species occasionally adapted itself to breeding in salt water. He found the larvae in salt pans as well as in air-raid precaution tanks and drums, etc. filled with sea water which had become diluted with rain water. Breeding was found on some occasions when the specific gravity of the water was equal to that of sea water. These should, however, be regarded as unusual situations. Unless disturbed the larvae feed on the surface like most other anophelines, but can dive into depths when disturbed (Iyengar, 1920). The larvae are also able to feed on suspended matter even at the bottom as well as on the sides of the water in large containers (Roy, 1931). Therefore, they are well adapted for breeding in deep waters such as in wells, etc.

A. stephensi larvae are, of course, shade lovers as they breed by preference in

deep wells into which sunlight penetrates only at midday or in wells permanently shaded and also in cisterns and overhead tanks.

Larval breeding in relation to bionomics. Intraspecific competition for food and space can be an important factor in the ecology of *A.stephensi* larvae. Because they grow in breeding places with limited surface or volume of water, they are subject to ecological pressures. Reisen (1975) studied this phenomenon. It was known from Roy (1931a) that there was reduced fecundity of females reared in the laboratory compared with wild ones. Reisen noted two opposite effects: (1) competition would contribute to the collapse of the overall population making individuals smaller and, with a reduced flight, imbibe less blood, produce fewer eggs and reduce survival, and (2) in permanent breeding sites such as 'seeps' and cisterns, the above mentioned attributes would help prevent larval densities from exceeding the carrying capacity of the habitat. Crowding would act as an internal regulatory mechanism delaying development and reducing fecundity, thus helping reduce the population growth when larval densities approached critical levels. It has been the experience of the present author that the larvae usually collected in wells are quite vigorous, healthy and well grown.

Relation to Disease

A. stephensi is one of the major vectors of malaria in India and in neighbouring countries of Pakistan, Iran and Iraq.

Dissections have been made in several localities in India and Pakistan. The first large series of dissections was in Bombay City (Bentley, 1911 quoted by Covell, 1927) when 91 gut and 30 gland infections out of 1,228 dissections were found. Some of the major series of dissection records are given in Table 36.

Table 36. Some dissections of *A. stephensi*

Name	Number dissected	No. of infections Gut	Gland	
Bentley 1911 (Reported by Covell 1927)	1,228	91	30	Bombay City
Covell 1928	671	17	12	Bombay City
Sweet & Rao 1931	2,710	2	—	Hiriyur Area Karnataka State
Mulligan & Baily 1936	719	5	2	Quetta, Beluchistan
Afridi & Majid 1938,	1,142	8	1	Bahrein Island, Persian Gulf
Siddons 1946	1,082	4/678	6/1052	Calcutta
Bhaskar Rao *et al.* 1946	902	1	1	Bellary, North Karnataka
Godbole *et al.* 1948.	1,272	—	1	Bijapur, Karnataka
Viswanathan 1950	4,706	—	1	Deccan, Maharashtra
Neogy & Sen 1962	1,613	—	1	Durgapur Steel Plant, Bengal

Other localities where positive specimens have been found are Delhi, Kohat (Pakistan), Sharanpur, Lucknow, Mopad (A.P.), Hyderabad City (Deccan), Khairpur State (Sind), Kutch (Gujarat), Visakhapatnam (A.P.) and Ahmedabad City and also in Iraq and Iran. While normally *A. stephensi* maintains a moderate degree of endemicity, it can also cause epidemics one of which was described by Banerjee (1930) in Lucknow.

The high prevalence of malaria in Bombay City, till Covell (1928) organized one of the finest programmes of *A. stephensi* control, is very well known.

In Calcutta, that *A. stephensi* had an important role was established by Siddons (1946). From 1942 to 1945, there was a definite increase in the local transmission of malaria which was attributed to *A. stephensi* type form. Among the anophelines he collected, he found 2,244 *A. stephensi* adults with only one *A. sundaicus* and one *A. culicifacies*. This is a remarkable observation in view of the well known occurrence of *A. sundaicus* in that city at that time.

Neogy & Sen (1962) considered *A. stephensi* to be a vector in rural Bengal also having found one gland positive out of 193 dissected in Damodar Valley area of Burdwan District. They did not find any positive in six other species dissected including 63 *A. culicifacies*. Also in Durgapur Steel Plant in rural Bengal, they found *A. stephensi* as the vector; out of 1,613 dissected they found one gland positive. They pointed out that *A. culicifacies* occurred in low densities and with *A. philippinensis*, the recognized vector in deltaic Bengal gradually disappearing, the role of *A. stephensi* assumed some importance in that region.

In coastal Orissa, Senior White had made a statement that it was not *A. sundaicus* but *A. stephensi* which was the chief vector in some of the coastal localities. It is well known that in North Andhra Pradesh in Visakhapatnam many positive dissections of *A. stephensi* have been made.

The work of Bombay State wokers, reported by Viswanathan (1950), as well as of Bhaskar Rao *et al.* (1946) in Bellary (Karnataka) has shown that *A. stephensi* was a vector of some importance in rural areas of Deccan. It had, however, a comparatively low infection rate, but it made up for it by occurring in large numbers. As already pointed out it was the most abundant anopheles species in the Maharashtra Deccan. In this general area, it shares the transmission with *A. culicifacies* and *A. fluviatilis*. The form in this area is *mysorensis* as determined by egg measurements by the present author.

In Madras City, where malaria was still persisting in 1978-79, the chief vector was as regarded. *A. stephensi* breeding in wells. Several other cities and towns in Tamil Nadu like Salem and Erode are well known places in which *A. stephensi* is a vector and effective control has not yet been established.

In one such city, Reuben and colleagues have made a comparative study of the susceptibility to infection with human malaria plasmodia between *A. stephensi* and *A. subpictus*. While *A. stephensi* was readily infected and over 50 per cent became infected, none of the *A. subpictus* became infected Das *et al.* (1979).

The role of *A. stephensi* in towns like Bangalore, Pune, etc. has undergone a

change. Though the species occurs it does not seem to be playing any part in malaria transmission now.

However, in many towns in Gujrat, the species is still very active in transmission. Broach town has been studied carefully by Nair and Samnotra (1964). They dissected in 1963-64, 87 females out of which they found one specimen with both gut and gland infection. They also made a study of the relation of malaria prevalence to *A. stephensi* densities in that city. The heavily malaria affected localities showed an adult density of 42 per man hour, while the less affected and non-affected localities showed densities of 8.0 and 0.33 per man hour respectively. *A. culicifacies* occurred in negligible numbers in the affected localities.

In Pakistan, Iran, Iraq and a number of countries to the west of India *A. stephensi* is an important vector. In Iran Al Tikrity (1964), describing the geographic distribution of the vectors has stated that *A. stephensi* is the main vector, in the alluvial plains in central and southern regions.

Knowles and Basu (1943) made an extensive series of experiments on the survival and susceptibility of *A. stephensi* to plasmodium infections. In controlled conditions of humidity and temperature they found that temperature appeared to be a more important factor than humidity. The percentage of survival was found to be highest at low temperatures when combined with higher percentages of relative humidity. Spring and hot weather conditions of Calcutta were most unsuitable for infections in this species. Experimental infections were heavy in conditions simulating those of the monsoon, post monsoon and cold weather. At 50° F temperature and all degress of relative humidity between 50 and 100 per cent there was no infection. A 100° F temperature was not favourable as no specimen survived long enough to become infective. However, between 60° and 90°, infections with *P. vivax* occurred. The heaviest salivary gland infections were obtained at 80° F. The complete development of *P. vivax* upto the sporozoite stage took 18 days at 60° F, 16 days at 70° F, 11 days at 80° F and only 9 days at 90° F. With *P. malaria* only gut infections were found between 70° F and 80° F. Complete development of *P. falciparum* took 14 days at 70° F, 10 days at 80° F and 9 days at 90° F. The infectivity of mosquitoes was also determined by the maturity and numerical density of gametocytes in the donor's blood.

In an experimental study Raghavan and Krishnan (1949) found that *A. stephensi* was refractory to infections by *Brugia malayi*, but Lahiri *et al.* (1972) were able to demonstrate the development of *Brugia ceylonensis* Jayawardane, in a *A. stephensi*.

Status of *A. stephensi* and subspecies/variety *mysorensis*. The marked difference in the habits of *A.stephensi* in many parts of India noted by early observers had led to the postulation of the existence of biological races. Among those who paid some attention to this matter were Mulligan and Baily (1936) in Quetta and Ramsay and Macdonald (1936) in Bengal. Rao, Sweet and Rao (1938) working in the then Mysore State noticed for the first time certain differences in the eggs both in measurements of length and breadth, and in the number of ridges on the floats. They named one of the varieties as *mysorensis*. The two forms were given a subspecies status by Puri (1949) and it was adopted by Stone *et al.* (1959), but

Knight and Stone (1977) have reduced it to just a synonym. The eggs of *A. stephensi* type were longer and broader than those of *mysorensis* and had definitely more number of ridges on float (18 average vs. 13 average). Details have been given earlier. The study of eggs for detecting morphological differences was greatly influenced by the classic studies on the eggs of *maculipennis* complex by Hackett, Missiroli and others in Europe.

Senior White (1940) examined the *A. stephensi* population of Calcutta city from the point of view of the possibility of the existence of two races and stated that both the type form and *mysorensis* occurred and that in recent years the type form had suddenly become rare. He found slight but insignificant differences in the maxillary indices. It appeared that the fertility of the *mysorensis* form in captivity was very low. In Calcutta both forms were attracted to cattle rather than to man.

Afridi and co-workers were able to distinguish the existence of the two races in Karachi, by egg measurements.

The validity of any biological taxon is to be determined on genetic basis. Not only should the form breed true in succeeding generations but there should also be a definite degree, even if not complete, of genetic isolation and incompatability with related forms. Cross mating experiments, if feasible, can provide very useful information.

Sweet *et al.* (1938) conducted such studies and observed that there was a definite incompatability. When crosses between the two forms were made, both ways, most females did not lay eggs. A small proportion did so, but the majority of the eggs were infertile. From the few fertile eggs, the next generation of adults was raised but they continued to exhibit the sterility of the hybrids. Many hybrid females had undeveloped ovaries. Therefore, it seemed that on genetic basis also the two forms were distinct.

Observations on the behaviour of the two forms in nature have generally supported the hypothesis. The type form had largely been (almost exclusively) found in urban areas, i.e. Bombay, Bangalore, Pune, Ahmedabad, Broach, Delhi, Madras, Salem, etc. and the *mysorensis* form predominantly in rural areas.

Though at the time of the studies by Rao & Sweet, they had felt that only the type form was an efficient vector, it has now been found that *mysorensis* is also a vector in several areas particularly in the Deccan plateau. It is also found to be a vector in Iran. It should be noted that formerly it had been believed that the *mysorensis* form did not occur in any area west of Pakistan, but it has now been recognised that it is widely prevalent in Iran and neighbouring countries. It had generally been accepted that it is easy to colonize the type forms, but not easy to colonize *mysorensis*. But the latter has also been colonized (Russell and Mohan, 1941). Subsequently colonies of *mysorensis* have been established in other places.

Davidson and Jackson (1961) have however shown that crossing between the two forms yield fertile offspring. Colluzi *et al.* (1970a) noted similar results in many crosses among strains from Iran, Iraq, India and Pakistan.

In making a comparative study of *A. stephensi* from three different localities. India, Iran and Iraq, Rutledge *et al.* (1970) made some interesting observations.

They were all from colonies which had been established for many years but replenished by fresh specimens from the parent colonies. First an analysis of the egg measurement showed that the three strains were intermediate between the type form and *mysorensis* with respect to egg length, but they more resembled *mysorensis* with respect to egg width and number of ridges on the floats and type form in respect of oviposition. The Iran strain resembled *mysorensis* with respect to laboratory feeding response, and adult longevity, but the Iraq and Indian strains more nearly resembled the type form in these respects. The Indian and Iraq forms were similar. The Iran form was more exceptional in the number of ridges on float, laboratory feeding response, susceptibility to infection by *Plasmodium cynomolgi,* and adult female longevity. Six reciprocal crosses were made and an intricate pattern of inter-strain fertility was observed. While increased fertility was noticed with some cases, decreased fertility was observed in other. Similar results were seen with F_1 hybrids also. Oviposition rates did not vary greatly and no abnormal gonadal development was noticed. Discussing these findings they concluded that both forms occurred widely distributed in the Indian sub-continent, but the population of a given locality was usually either one or the other, the former being chiefly urban and the latter chiefly rural. It seemed unlikely that cryptic species existed within the taxon *A. stephensi* since inter-breeding is possible among all forms tested so far. In addition a sub-species status seemed inappropriate since the two forms were sympatric. Thus it was concluded that on the basis of present information it would be best to regard these and other forms as local population varieties.

Ashraf and Aslamkhan (1973) in Pakistan made a study of the measurements like the number of ridges to find out variation within the eggs of a single female. 25-50 eggs of 50 individual females from the Karachi strain, the so called *A. mysorensis,* were measured. On studying 1,712 eggs it has been found that length and breadth of eggs within a female are highly variable. Furthermore, the range covers the size of eggs of both *stephensi* and *mysorensis.* The measurements are as follows:

Length	505.59	2.82	microns
Range	440.00	585.00	"
Breadth	175.79	1.43	"
Range	130.00	225.00	"

It appeared to them that creation of subspecies categories in *A. stephensi* on the basis of morphology of eggs was not justified.

Cytotaxonomy is a modern tool which can explain many intricate problems of speciation. Detailed maps of salivary gland chromosomes have been made of *A. stephensi* by Sharma *et al.* (1969) but they describe only the type form. A recent study (Siddiqi and Aslamkhan, 1973) seems to indicate that an inversion has been recognised in the polytene chromosomes of *A. stephensi mysorensis.* Therefore while epidemiologically there is much support for the existence of two forms of *A. stephensi,* their true genetic and cytotaxonomical status needs still to be unravelled.

Studying chromosomal inversions in *A. stephensi.* White (1974) has pointed out

that certain aspects of morphology and behaviour were associated with them.

Knight and Stone (1977) in their *Catalog of the Mosquitoes of the World* have now listed *mysorensis* as a synonym, following the findings of Rutledge *et al.* (1970) but it is being treated as a variety in the present publication, in view of the now well recognized differences in behaviour.

Control

The control of *A. stephensi* has received considerable attention from quite early days after Ross's discovery of the role of mosquitoes in the transmission of malaria. Malaria in the major cities of India being known to be caused by it, the species became practically the first to be successfully controlled. Before the variety *mysorensis* was recognized, it had been assumed that *A. stephensi* was predominantly an urban mosquito with predilection for breeding in man-made breeding places. Bombay City was among the first to have an anti-*stephensi* programme. The details of the programme have already been dealt with in an earlier chapter. The successful programme in Bombay has been followed with good work in several other cities such as Delhi and Bangalore. However, the species has not responded to the control measures, mainly directed to control breeding in domestic wells, in certain urban areas such as Salem, Madras, etc. in Tamil Nadu and even in recent years in the vastly growing metropolis of Delhi. The problem appears to be one more related to the development of proper organization and management rather than to technical reasons. Use of larvivorous fish has remained the main measure to rely but in recent years chemical larvicides, particularly temephos (abate), a long lasting insecticide, has come into use. Used at the proper dosage and with precautions it can give good control of the larvae in wells without hazards to man and domestic animals. However, management of wells by elimination, screening, provision of hand-pumps after covering the wells, and similar measures with thorough periodical inspections and corrective actions can alone bring about permanent results. Indiscriminate storage of water in cisterns, barrels, cement tanks, etc. during urban house construction has to be strictly regulated. The urban *stephensi* problem is not insurmountable given the will and drive.

The problem posed by the rural *stephensi* however, is one as difficult as that of other major vectors. While initially there were good results in common with other vectors with which the species is usually associated, the results in recent years have not been satisfactory. It cannot be estimated as to how much of the recrudescence of malaria is due to rural *stephensi*, as there have not been any concerted programmes of mosquito dissections. But the species is now known to have developed resistance to DDT or HCH in many places in India and countries to the west and consequently the prospects of control by insecticides are not very bright. The control of rural *stephensi* needs the same type of attention and strategy as does of *A. culicifacies* with which it breeds in common breeding places.

Anopheles theobaldi Giles, 1901

Type locality: Ellichpur, Amaraoti District, Maharashtra.

Type: British Museum.

Taxonomy: Closely related to *A. maculatus.*

Distribution: India, Nepal, Pakistan, Burma, probably also Bangladesh.

In India: Found scattered in many localities throughout India, but mainly concentrated in the central and western parts of the peninsula.

Recently, Ramachandra Rao *et al.* (1973) have found the species in many localities in the U.P. and West Bengal, Himalayas, such as Nainital, Pithorgarh, Chamoli and Darjeeling Districts. Viswanathan (1950) records it practically from all areas of old Bombay State, except in Gujarat.

Distinguishing characters: A medium sized mosquito, resembling *A. maculatus* but distinguished by absence of dark band on segment 4 of hind tarsus; last two segments of hind tarsi completely white; foretarsi only apically banded. Larva very similar to *A. maculatus.* Reid, Wattal and Peters (1956), who studied *A. maculatus* and allies in India, think that *theobaldi* may be only a polymorphic variety of *A. maculatus*, differing from it only in the absence of dark band on hind tarsal segment 4. Reid (1968) has tentatively accepted its status as a species, but if later it is decided to be a variety, the name *theobaldi* has priority over *maculatus.*

Ecology/Bionomics: What little is known indicates that the habits and habitats are like those of *A. maculatus.*

Relation to disease: No evidence of role in malaria transmission though many dissections have been made, but in small numbers. To Wattal's (1961) list must be added 131 dissections made by the Bombay workers (Viswanathan, 1950), all with negative results, mainly in Jalgaon District (East Khandesh).

APPENDICES

Appendix I

Keys for Identification of Indian Anophelines

Keys such as those given below are solely for purposes of rapid identification of different species for routine purposes. Only one or two very distinctive features which can be seen with a hand lens or a low powered microscope are selected and employed. Some of them may be absolute characters and others which occur in the majority of individuals. The keys do not in any way supplant detailed study of all parts of the specimen for accurate identification. Between closely related forms the distinction depends on a combination of several characters and not by a single character which may also be variable. Therefore, students of mosquito entomology should appreciate the limitations of keys and when making critical studies of individual specimens or their distribution, examine them with greater thoroughness or if necessary, send them to recognized taxonomic experts.

Many variations within the same species have been frequently noticed, either due to season or geographical distribution. When such variations are noticed, detailed publications on taxonomy should be consulted. Wattal *et al.* (1960) have given a good description of the several types of variations which occur in Indian anophelines.

Keys to Indian *Anopheles* species have been published by many authors, chief among whom are those of Central Malaria Bureau (1912), Strickland and Choudhury (1927), Knowles and Senior White (1927), Christophers, Sinton and Covell (1927), Christophers (1933), Puri (1949 and 1960), Wattal (1963), Roy and Brown (1970), Wattal and Kalra (1961) and others. The most useful to Indian workers have been the illustrated keys published in the Health Bulletin (No. 10) by the Government of India. International workers, such as Russell, Rozeboom and Stone (1943), Reid (1968), Harrison and Scanlon (1977), Peyton and Scanlon (1966), and others have published comprehensive keys for the Oriental anophelines. An atlas of the malaria vectors prepared by Ross and Roberts (1943) was published by the American Entomological Society (1943).

The question whether keys should be included or not in the present book was carefully considered and it was decided that simple keys for adults larvae, and eggs may be provided. Pupal keys have not been given as they are still far from useful for routine purpose. Those interested should refer to the detailed taxonomic works, such as Christophers (1933), Reid (1968), Harrison and Scanlon (1975), etc.

The keys presented below are largely based on those of Christophers (1933) with several modifications. The author is grateful to the Director, Zoological Survey of India, Calcutta, for kind permission to utilize Christophers key as the basis.

Wherever several species have been bracketed, the further distinguishing characters are given under the main species of the group.

No originality is claimed for the keys.

For the newer species and for a few species of other countries included in the key reliance has been placed on the detailed descriptions given by taxonomic workers on Southeast Asian anophelines.

A. Adults

I. Wings completely dark or if pale areas present, dark areas on costa involving both costa and vein 1 are less than four in number; parabasal spines of male two; pharyngeal armature absent. — II Subgenus *Anopheles*

Wing always with pale and dark markings; dark areas on costa involving also vein 1 four or more in number; parabasal spines of male four or five in a group. — III Subgenus *Cellia*

II. **Subgenus Anopheles**

1. Wings entirely dark. — 2
   Wings with pale markings. — 4
2. Hind femur with distinct white knee spots at at distal end. — *barianensis* p. 235
   Hind femur without such a knee spot. — 3
3. Head scales very narrow, rod like. — *aitkenii* p. 230; *bengalensis* p. 232; *insulaeflorum* p. 233; *pinjaurensis* p. 233
   Head scales of ordinary type. — *culiciformis* p. 234; *sintoni* p. 235
4. Hind femur with an outstanding tuft of white and black scales at its distal end, visible to the naked eye. — *annandalei,* p. 240; *interruptus* p. 240
   Hind femur not so. — 5
5. Hind femur with a broad white band. — *lindesayi* and subspecies *nilgiricus* p. 236
   Hind femur without such a band. — 6
6. Inner quarter of costa pale. — gigas P. 237 and its varieties; *simlensis* p. 238; *baileyi* p. 239; *refutans* (Sri Lanka only)
   Inner quarter of costa mainly dark though there may be a few scattered pale scales. — 7

7. Palpi with definite pale markings; clypeus with tuft of black scales at side.	*nigerrimus*	p. 242
	sinensis	p. 250
	argyropus	p. 241
	crawfordi	p. 242
	nitidus	p. 248
	pediataenitus	p. 249
Palpi without any pale markings.	8	
8. Females with prominent tuft of scales on ventral side of abdominal segment VII; inner third of costa with a few pale scales.	*barbirostris*	p. 253
	ahomi	p. 253
	barbumbrosus	p. 259
	campestris (not recorded in India).	
Females without such a tuft. Inner third of costa without pale scales.	*umbrosus*	p. 261
	roperi	p. 260

III. **Subgenus Cellia**

1. Tip of hind tarsi not white.	2	
Tip of hind tarsi white.	17	
2. Tarsi of front legs with broad pale bands.	3	
Tarsi of front legs unbanded or only with narrow bands.	6	
3. Femora and tibiae speckled.	4	
Femora and tibiae not speckled.	5	
4. Female palpi with both apical and preapical pale bands broad and one narrow more basal band, sometimes speckled; thorax with broad scales.	*stephensi*	p. 410
Female palpi with only the apical pale band broad; preapical band narrow; thorax not covered with broad scales.	*sundaicus*	p. 371
5. Palpi of female with dark preapical area equal to or nearly equal to the pale apical band;*	*subpictus* and var. *vadakadiensis*	p. 361
Palpi of female with dark preapical area half or less than half the length of the apical broad band;*	*vagus*	p. 369
6. Thorax with obvious scales.	7	
Thorax with hairs or hair-like scales only.	11	
7. Tip of female palpi dark.	8	
Tip of female palpi not dark.	9	
8. Fossae in both sexes covered with scales.	*multicolor*	p. 381
Fossae devoid of scales.	*turkhudi*	p. 381
9. Tarsi with narrow but distinct pale bands.	10	
Tarsal bands absent or indistinct and not white.	*superpictus* (not recorded in India)	

*At present males are indistinguishable.

10. A line of overlapping broad white scales on side of thorax in front of wing roots.	*moghulensis* p. 398	
Without such a line of scales, scaling confined to median area of dorsum of thorax.	*jeyporiensis* and var. *candidiensis* p. 357	
11. Spotting of wings confined to costa and vein 1 only, rest of the wing dark; head scales narrow and rodlike.	*dthali* (Kashmir only) p. 357	
Wing with usual wing spots on all veins; head scales or ordinary type.	12	
12. Female palpi with two broad pale apical bands, as broad or broader than the intervening dark area.	13	
Female palpi with subapical pale band narrow; intervening area very broad.	15	
13. Fringe spot present on vein 6; apical half of proboscis pale.	*aconitus* p. 278	
No fringe spot on vein 6; proboscis dark or apical half pale in certain lights only.	14	
14. Basal third of costa uninterruptedly dark, without pale interruption, not even with a pale scale; outer half of proboscis faintly or more markedly pale in certain lights.	*varuna* p. 311	
Basal third of costa with a pale interruption however small; proboscis with apical half dark; except sometimes with a pale spot ventrally.	*minimus* p. 298	
15. Fringe spots well marked at all veins except 6; some erect pale scales in front of thorax; vein 1 pale in basal area.	16	
Fringe spot on two veins only except rarely; no pale scales or very few in front of thorax; vein 1 internal to inner dark costal spot with dark spot (Vein 1 with a dark spot opposite the pale interruption on costa outside humeral cross vein); third vein mostly dark.	*culicifacies* p. 317	
16. Third vein usually extremely pale; thorax with median area markedly paler than dark sides; frontal tuft conspicuous. (Inner quarter of costa entirely dark)	*fluviatilis* p. 280	
Third vein all dark or with only a pale spot; thorax uniformly coloured; frontal tuft poorly developed.	*sergentii* (Not found in India)	
17. Hind tarsi with only one segment or less white, commonly with white bands above this.	18	

Hind tarsi with a continuous white area embracing *atleast* two terminal segments.	23		
18. Femora and tibiae not speckled.	19		
Femora and tibiae speckled.	20		
19. Female palpi with two broad pale apical bands and one narrow band near base in addition to usual more basal band (total 4 bands)	*karwari*	p.	392
Female palpi with two broad pale apical bands and the usual basal only (Total 3 bands)	*majidi*	p.	360
20. Sixth vein with not more than three dark spots.	21		
Sixth vein with more than three dark spots.	22		
21. Abdomen with a row of continuous black scale tufts on ventral surface of all segments easily visible in the lateral view to naked eye; female palpi with four pale bands.	*kochi*	p.	274
Abdomen not so, female palpi with usual three bands only.	*maculatus* and var. *willmorei*	p.	393
22. Tibio-tarsal joint of hind leg with a broad conspicuous white band.	*balabacensis* and *elegans*	p.	263, 273
Tibio-tarsal joint of hind leg without a broad band.	*tessellatus*	p.	276
23. Femora and tibiae not speckled. Femore and tibiae speckled.	24 26		
24. Hind tarsi 3¾ segments continuously white; abdomen heavily clothed with broad scales, which form lateral tufts except on the last few segments.	*pulcherrimus*	p.	407
Hind tarsi 3½ or less segments continuously white; abdomen with almost rather narrow scales not forming tufts except on last few segments.	25		
25. Vein 5 mainly dark; or with atleast a dark spot about the middle near the origin of the branch.	*annularis*	p.	383
Vein 5 continuously pale except at base and apex.	*pallidus*, *philippinenensis* *nivipes* (not in India)	p.	399, 401
26. Hind tarsi with only two segments completely white.	*theobaldi*	p.	425
Hind tarsi with three segments completely white.	27		
27. Female palpi with two broad apical pale bands and a narrow band and conspicuous speckling; male palpi with shaft banded and spotted with white.	*splendidus*	p.	409
Female palpi with broad apical pale bands or			

two narrow pale bands, without speckling; male palpi with shaft dark. 28

28. Dorsum of last two abdominal segments clothed with golden hairs and scales; inner quarter and outer third of costa chiefly pale. *jamesii* p. 391
Dorsum of last two abdominal segments not so, inner quarter of costa and outer third of costa chiefly dark. *ramsayi* p. 408

B. Larvae

NOTE: Only fully grown fourth stage larvae should be examined under a microscope. (IC—inner clypeal hairs; OC—outer clypeal hairs; PC—posterior clypeal hairs)

I. IC more or less close together, the distance between their bases never more than between bases of IC & OC of one side. Antennal hair usually branched (except in a few tree-hole breeding species). II Subgenus *Anopheles*
IC well separated, the distance between their bases twice or more than distance between IC & OC on one side. Antennal hair simple (very rarely bifid or trifid). III Subgenus *Cellia*

II. **Subgenus Anopheles**

1. Antennal hair simple, on dorsoexternal surface of antenna; most frontal hairs simple and short. 2
Antennal hair branched, arising from internal surface of antenna; frontal hairs always long and branched. 5
2. OC branched; body-integument covered with innumerable conspicuous minute recurved setae. 3
OC simple; body-integument not covered with such setae. 4
3. Both long pleural hairs on each side of meso- and meta-thorax simple. *annandalei*
One long pleural hair on each side of meso-and meta-thorax barbed sparsely. *interruptus*
4. Bases of IC not close together; all frontal hairs very short and simple; thoracic palmate hair not differentiated. *barianensis*
Bases of IC close together nearly touching each other; thoracic palmate hair fairly well developed. *culiciformis, sintoni*

5.	OC simple, bifid or with a few short primary branches.	6
	OC many branched forming a tuft.	12
6.	IC split about the middle into 2-5 branches, bases not close together.	7
	IC simple, bases nearly touching.	8
7.	IC split into two a little above base.	*aitkenii*
	IC split about the middle into 3-5 branches.	*bengalensis*
8.	Palmate hairs on thorax and abdominal segments I-VII present or on II-VII fairly well developed; all clypeal hairs simple.	9
	Palmate hair not differentiated on thorax nor on any abdominal segment or only on III-VII; OC & PC may be branched.	10
9.	OC about 1/3 length of IC.	*insulaeflorum*
	OC about 1/2 length of IC.	*lindesayi* and subspecies *nilgiricus*
10.	Palmate hair not differentiated on thorax or any abdominal segment. OC split into 5-11 & PC into 2-3 branches.	*umbrosus*
	Palmate hairs only on abdominal segments III-VII; not differentiated on thorax; OC & PC not differentiated on thorax; OC & PC may be branched.	11
11.	OC branched (2-6); PC simple	*gigas* and some of its varieties.
	OC simple; PC usually into 2-5 branches.	*gigas* var. *simlensis*
12.	Inner shoulder hair (IS) of prothorax with branches arising near base.	13
	IS simple or with distal end only split into 2 or 3 branches.	*"hyrcanus"* group See under *nigerrimus*
13.	IC completely simple	*barbumbrosus, barbirostris*
	IC frayed.	*ahomi* see under *barbirostris*

III. **Subgenus Cellia**

1.	Anterior tergal plates on abdominal segments III-VI very large with convex posterior border and enclosing the small posterior plates.	2 *"minimus"* group
	Anterior tergal plates on abdominal segments III-VII not exceptionally large and not enclosing the posterior small tergal plate.	4
2.	All clypeal hairs simple.	3
	OC & IC with short scattered branches; PC	

branched from base.	*aconitus*
3. A pair of minute hairs (hairs 0) arising from tergal plate.	*varuna*
Hair O not arising from tergal plate but posteriorly outside it.	*minimus, fluviatilis*
4. IC and OC simple or with short inconspicuous lateral branching.	5
IC and OC with lateral branches or conspicuously frayed.	17
5. Fairly well developed palmate hairs on abdominal segments I-VII; both long pleural hairs on meso-thorax simple (one of which may be bifid)	6
Fairly well developed palmate hairs on abdominal segments II-VII; one long pleural hair on meso-thorax pectinate, the other simpler.	12
Fairly well developed palmate hairs on III-VII; both long meso-and meta-thorax pleural hairs or each side simpler.	16
Fairly well developed palmate hairs only on IV-VI and comparatively very small, both long mesothoracic pleural hairs feathered; PC about as long as IC.	*turkhudi*
6. All thoracic pleural hairs simple; filaments of abdominal palmate hairs blunt.	*kochi*
Some of the thoracic pleural hairs pectinate; filaments of abdominal palmate hairs sharp pointed.	7
7. Palmate hair not differentiated on thorax; root of inner shoulder hair inconspicuous; both long metathoracic pleural hairs pectinate; all prothoracic pleural hairs simple; or one of them may be split into 2 or 3.	8
Palmate hair on thorax more or less differentiated; base of inner shoulder hair more or less conspicuous and brownish; one long metathoracic pleural hair pectinate, the other simple, one long prothoracic pleural hair feathered.	9
8. OC and PC about 1/3/(or less) as long as IC; PC internal and close to IC.	*vagus*
OC and PC not so short.	*subpictus, sundaicus* (See under *sundaicus*)
9. Root of inner shoulder hair is poorly developed; one long metathoracic pleural hair pecti-	

nate and other simple.	*sergentii* (not in India)
Root of inner shoulder hair thickened and conspicuous; both long mesothoracic pleural hairs simple.	10
10. IC exceptionally long (about 1/2 the length of fronto-clypeus); filaments of abdominal palmate hair about 1/4 the length of blades of leaflets.	*majidi*
IC normally long (much less than 1/2 the length of fronto-clypeus); filaments of abdominal palmate hair about half or more than as long as blades of leaflets.	11
11. PC about half the length of OC; branches of inner submedian caudal hair slender and their ends straight.	*culicifacies*
PC about as long as OC; branches of inner caudal hair like those of the outer and hooked.	*dthali*
12. Filaments of abdominal palmate hairs about 2/3 or as long as blades; thoracic palmate hair fairly well developed (except in *multicolor*)	13
Filaments short; 1/2 or less than length of blade; thoracic palmate hair not well developed.	15
13. IC and OC slender PC longer than OC; palmate hair of abdominal segment II smaller than those on following segments.	14
IC and OC stout; PC a little shorter than OC; palmate hair on abdominal segment II as large as on the following.	*moghulensis*
14. IC and OC faintly frayed; palmate hair on metathorax fairly well developed.	*superpictus*
IC and OC simple; palmate hair on metathorax not developed.	*multicolor*
15. OC always somple, IC usually so; palmate hair never differentiated on metathorax.	*stephensi*
OC and IC finely frayed; palmate hair on metathorax may be slightly differentiated.	*theobaldi, maculatus* and var. *willmorei* (in part)
16. IC with only 2-4 branches and arising from an inconspicuous root; poorly developed palmate hair on metathorax.	*tessellatus*
IC with numerous branches, with a large brown root; palmate hair on metathorax not at all differentiated.	*balabacensis, elegans*

17: OC with long branches, often about as long as the hair itself. 18
OC with short lateral branches never more than 1/6 length of the hair. 22

18. OC with many branches forming a broom like tuft. 19
OC with 4-12 distal branches only. *pulcherrimus*

19. Inner sutural hair simple or bifid near top. 20
Inner sutural hair split near base into 2-8 branches. 21

20. Palmate hair on abdominal segment I well developed; (larvae usually dark grey with two or three silvery spots on dorsum). *annularis*
Palmate hair on abdominal segment I not differentiated; usually pale dirty yellow colour. *jamesii*

21. PC with 2-5 branches; filaments of abdominal palmate hairs about half or more as long as blades of leaflets. *pallidus*
PC with 7-10 branches; filaments about 1/4 long as blades. *philippinensis*

22. OC pinnate, with large number of branches arising practically the whole length; one long pleural hair on metathorax simple; well developed palmate hairs on metathorax and on abdominal segments I-VII. (Anterior tergal plate rather large). *jeyporiensis*
OC with only a few scattered branches; both long pleural hairs on metathorax feathered. (Anterior tergal plate small) 23

23. OC exceptionally long (about half as long as fronto-clypeus); shortest pleural hair in prothoracic group normal, with 2-4 branches; palmate hair on abdominal segment II poorly developed. *ramsayi*
OC normally long; shortest pleural hair on prothoracic group normal with 2-4 branches only; fairly well developed palmate hairs on abdominal segments II-VII. 24

24. OC often split into two, and with 3-7 short inconspicuous branches; inner sutural hair split into 2-4; filaments of abdominal palmate hairs very broad at base with blunt end and about 1/2 length of blade. *splendidus*
OC with a few five lateral branches; inner sutural hair simple; filament not very broad; but

may be blunt or sharp pointed.	25
25. Lateral hair on abdominal segments V & VI with 6-10 long branches like a pectinate hair; filament of palmate hairs blunt; palmate hair on metathorax never differentiated, but the corresponding hair with 4-9 lateral branches like a pectinate hair.	*karwari*
Lateral hair on abdominal segments V & VI splitting near base into 3-5 branches; filament of palmate hairs usually sharp pointed, palmate hair on metathorax usually not differentiated but hair corresponding to it splits with 2-6 branches (in some cases slightly flattened).	*theobaldi, maculatus* and var. *willmorei* (in part)

NOTES:

1. It would be well to remember that there is a considerable range in the variations in the branching of hairs. As far as possible, one should examine a number of characters before deciding as to the species.
2. Several newly recognized or reconfirmed species occurring in India do not appear in the above keys largely drawn from those of Christophers (1933) based on Puri's (1931) monumental work. Very little attention has been given to anopheline larval chaetotaxy in India during the last four decades, except a few stray appears all of which have been referred to in the body of the main text. Differences between closely related species or recently split species are also described in the text.

   Species which do not find reference in the larval keys are:

   crawfordi, roperi, argyropus, nitidus and *pediataeniatus.*

   Readers are advised to refer to books like those of Reid (1968), Peyton and Scanlon (1966) and Harrison and Scanlon (1975).

C. Eggs

A.	Lower surface with polygonal marks.	
	1. Floats absent.	*barianensis*
	Floats present.	2
	2. Floats touching margin of upper deck.	*annandalei, lindesayi*
	Floats not touching margin of upper deck.	*nigerrimus, barbirostris gigas, tessellatus, kochi, varuna*
B.	Lower surface without polygonal marks:	

1. Floats absent.	*turkhudi, multicolor, superpictus dthali*
Floats present.	2
2. Floats touching margin of upper deck.	3
Space between deck and float very narrow.	*aitkenii*
Floats clearly separated from upper deck.	6
3. Frill continuous along whole margin of upper surface.	*subpictus, vagus, sundaicus, pulcherrimus*
Frill interrupted in middle.	4
4. Upper surface as wide as egg body; no part of the lower surface visible from above.	*annularis, pallidus, jeyporiensis, philippinensis*
Upper surface narrower than the egg body; some portion of the lower surface visible from above.	5
5. Floats distinctly nearer the narrower end.	*ramsayi, jamesii moghulensis, sergentii.*
Floats equidistant from either end.	*maculatus, stephensi, karwari, splendidus.*
6. Frill well developed in the middle part.	*culicifacies.*
Frills discontinuous or very narrow in the middle part.	*fluviatilis, aconitus, minimus.*

NOTES:

1. The above key is mainly based on the keys provided by Christophers and Barraud (1931) and Christophers (1933). The descriptions of eggs from Sri Lanka by D'abrera (1944) have also been useful.
2. Description of eggs of the other anophelines species have not been available to the author.
3. Eggs vary considerably in their sizes and relative measurements, as also in the number of ridges present on the float. In some cases the upper deck is divided into two, as in *A. philippinensis, A. maculatus* and sometimes in *A. fluviatilis.*

Appendix II

Entomological Techniques

Every entomologist dealing with mosquito vectors of diseases has to be fully familar with the special techniques which have been developed in respect of the vectors of particular diseases he is dealing with. It is beyond the scope of this book to describe them. Mention can, however, be made of some of the most useful and upto-date publications which the workers should consult. Among them are:

1. *Manual on Practical Entomology in Malaria,* in two parts, published by the World Health Organisation, 1975.
2. *Practical Malariology,* by Russell, P.F., West, L.S., Manwell, R.D. and Macdonald, G. Oxford University Press, London, 1963.
3. *Mosquito Ecology, Field Sampling Methods,* by N.W. Service, Applied Science Publisher, London 1976.
4. *A Practical Entomological Course for Students of Malariology* by Barraud, P.J., (1937) revised by Puri, I.M. Health Bulletin (Malaria Bureau Bulletin) No. 9, Government of India, Manager of Publications, Delhi.

Of these the WHO publications referred to above are the most detailed, up-to-date and comprehensive. They have been prepared primarily for use of workers on malaria.

Workers on filaria entomology can adopt the above techniques for most of the field studies except in regard to dissections for filaria infections of mosquitoes, for which they would have to refer to general textbooks on parasitology.

Studies on arboviruses require a deep understanding of the behaviour and distribution of vectors including anophelines. Specialised information on mosquitoes, processing of mosquitoes for virus isolation can be obtained in standard works such as *Viral and Rickettsial Infections of Man* 4th Edition, Edited by Horsfall and Tamm, 1964. Detailed information on the techniques of collection and preservation of mosquitoes for virus isolation can be obtained from the Director, National Institute of Virology, Pune.

Unlike in malaria and filariasis, the field entomologist cannot ordinarily process the mosquitoes to determine the vector. Virus isolation involves the availability of infant mice or special tissue cultures. It would be best to send batches of mosquitoes to a well equipped laboratory specially working on arboviruses.

The special entomological techniques for malaria, filariasis and virus studies with which workers should be familiar are:

1. Collection, identification and preservation of mosquitoes of all stages.
2. Measurements of adult and larval densities by standard methods.
3. Studies on biting, resting, ovipositing and flight habits, etc.

4. Determination of breeding places and their relative importance.
5. Dissection of adult females for:
   (a) Plasmodial and filaria infections
   (b) Gonotrophic conditions/parity
   (c) Age determination
   (d) Insemination
6. Tests for susceptibility to insecticides, of both larvae and adults, by standard methods developed by the WHO and interpretation of data.
7. Maintenance of mosquito colonies for experimental purposes.
8. Collection of mosquito stomach bloods for tests for sources of blood meal (precipitin test).
9. Principles and practice of control of the adults and larvae in different circumstances.

Appendix III

Changes in Names of Places

During the last four or five decades, especially after World War II, many changes have occurred in the names of places in the entire Oriental region. Changes are still taking place, for example the most recent one being the change of "Cambodia" to "Kampuchea". Unless one is familiar with these changes, some confusion will arise when reading the earlier literature. Numerous changes in names of districts and towns have also occurred; so also have redistribution of areas into new states and districts taken place widely. It would be beyond the scope of this book to make a comprehensive list of the changes, but given below are some of the major changes which have occurred so far as they have relation to the present subject.

A. **Changes of place names in areas outside of India**

Former	*Present*
Pakistan (West):	Pakistan
Pakistan (East):	Bangladesh
Ceylon:	Sri Lanka
Siam:	Thailand
Malaya, Federated Malaya States:	Malaysia
North Borneo:	Sabah, Brunei, Sarawak.
Dutch East Indies:	Indonesia
Celebes:	Sulawesi (Part of Indonesia)
Borneo (except N. Borneo):	Kalimantan (Part of Indonesia)
Indochina:	Vietnam, Kampuchea (Cambodia), Laos
Formosa:	Taiwan
Dutch New Guinea:	West Irian (Part of Indonesia)

B. **Changes of place names of India**

After Independence the political map of India has undergone many major changes. Firstly, all Indian States (so called Native States) have disappeared and became parts of the major States of India. Secondly, in the formation of new States, mainly on linguistic basis, several States have been broken up and parts from several States or Provinces have been brought together into new States. Students of Indian anophelines who read old literature should make a note of these changes so that the earlier records may be appropriately referred to the modern States. The words such as 'Province' and 'Presidency' have also disappeared from use. Some areas such as Delhi, Goa, Pondicherry etc. are listed as Union Territories.

Names of States *New Names*	*Old names of Provinces, States and Territories*
Andhra Pradesh	Madras Province (northern part) and parts Hyderabad State.
Assam	Assam excluding several hill states
Bihar	Bihar and several Indian States
Gujarat	Northern parts of Bombay Province and Kathiawad (Saurashtra), Baroda and several Indian States.
Haryana	Part of Punjab.
Himachal Pradesh	Several Himalayan Indian States and Simla.
Jammu and Kashmir	Jammu and Kashmir
Karnataka	Mysore State, Coorg, parts of Bombay, Madras and Hyderabad States
Kerala	Travancore and Cochin States and parts of Madras Province.
Madhya Pradesh	
Madhya Pradesh	Major parts of Central Provinces and several Indian States in Central India such as Bhopal, Indore, Gwalior, etc.
Maharashtra	Southern part of Bombay Province, Vidarbha area of Central Provinces and Marathawada of Hyderabad State, and several Indian States.
Manipur	Manipur State
Meghalaya	Part of Assam
Nagaland	Part of "Assam" Hill area.
Orissa	Orissa, parts of Madras Province and several Indian States in east central India.
Punjab	Part of the Punjab and several Indian States.
Rajasthan	Rajputana states, including Jodhpur, Jaipur, Bikaner, etc.
Sikkim	Sikkim State
Tamil Nadu	Madras Province (southern parts).
Tripura	Tripura State
Uttar Pradesh	United Provinces
West Bengal	Undivided Bengal (western part) and Cooch Bihar.
Union Territories:	
Andaman-Nicobar Islands	Andaman-Nicobar Islands

Arunachal Pradesh	North-East Frontier Agency
Chandigarh	New City in old state of Punjab, now common capital of Punjab and Haryana.
Dadra and Nagar Haveli	Part of Goa in Gujarat region.
Delhi	Delhi
Goa, Daman and Diu	Part of old Goa (Portugese)
Lakshadweep	Laccadive Islands.
Mizoram	Part of "Assam" region.
Pondicherry	Pondicherry (French)

Names of some important towns and cities

Former	*Present*
Poona	Pune
Thana	Thane
Banaras	Varanasi
Baroda	Vadodra
Tanjore	Thanjavur
Trichinopoly	Tiruchirapally
Madura	Madurai
Cawnpore	Kanpur
Ootacamund	Uthagamandalam
Vizagapatam	Visakhapatnam
Bezwada	Vijayawada
Nagpore	Nagpur
Jubbalpore	Jabalpur

Appendix IV

Designation of Types

Every student of entomology should be familiar with the rules, conventions and practices of giving names to animal species. The International Code of Zoological Nomenclature, London, 1964, provides authoritative guidance in this regard.

A TYPE is a single specimen which is the basis for the description of a taxon, either the original specimen or one designated as such from the type series.

Some of the kinds of 'types' mentioned in mosquito literature are listed below. Those which find mention in the International Code are marked with an asterisk(*).

ALLOTYPE	A specimen of the opposite sex to the holotype designated by the author as a paratype.
HOLOTYPE*	If a nominal species is based on a single specimen this specimen is the 'holotype'.
LECTOTYPE*	If the nominal specimen has no holotype any one of the syntypes may be designated as lectotype.
NEOTYPE*	A specimen selected as a type after the original description if the original type, lectotype or syntype is lost or destroyed.
PARATYPE*	After designating a holotype all other syntypes are to be labelled as paratypes. After designating a lectotype all other syntypes are to be labelled as paralectotypes.
PLESIOTYPE	A specimen or specimens upon which subsequent descriptions or figures are based.
SYNTYPE	Every specimen in a type-series in which no holotype was designated.
TOPOTYPE*	A specimen collected at the type locality.

The word 'cotype' in the sense of 'paratypes' should not be used. The above definitions have been drawn from the International Code (1964) or from Mayr (1969, reprinted 1977).

Regarding genera, a 'LOGOTYPE' is a genotype selected subsequently to the first publication of a generic name.

Information on the Type Material of Indian Anophelines

Thanks to the courtesy and co-operation of several scientists in charge of museums and laboratories, some uptodate information has been collected on the location of most of the types of Indian anophelines. The following information, though not complete in every respect, is provided for the benefit of workers.

National Institute of Communicable Diseases, Delhi: Courtesy of Dr. B.L. Wattal: At the time of partition of India in 1947 the collections at the old Malaria Institute of India were distributed between the Indian and Pakistani sections. The current information as kindly furnished by Dr. Wattal is given below:

A. gigas var. *simlensis* James	None.
A. sintoni Puri	4 males and 3 females paratypes present, from Calicut, Malabar District.
A. pinjaurensis Barraud	1 male (type) present. Coll. Pinjaur.
A. aitkeni spp. *bengalensis* Puri	1 female paratype from Marianbari.
A. majidi McCombie Young and Majid	3 males and 3 females; type from Mercara, Coorg.
A. moghulensis Christophers	None.

No information is available regarding type of *A. subpictus* var. *vadakadiensis* Doraisamy, 1962, stated to be deposited in the 'National Malaria Institute., (Sic).

Zoological Survey of India, Calcutta: (Also internationally referred to as the Indian Museum). **Courtesy of Prof. T.N. Ananthakrishnan, Director:**

The type specimens of the following species are present:

Anopheles annandalei Prashad;

A. annandalei var. *interruptus* Puri (now raised to the status of a species);

A. lindesayi var. *maculata* Theobald (var. *maculata* is now considered merely as a synonym).

The type material of *Anopheles varuna* Iyengar, co-types (paratypes) of which were recorded as in the Indian Museum, are not present now in the ZSI collections.

British Museum (Natural History), London: Courtesy of Dr. G.B. White: The following type specimens are available in the British Museum.

Anopheles aitkenii James
A. barianensis James
A. culiciformis Cogill
A. gigas Giles
A. gigas var. simlensis James
A. indiensis Theobald
A. karwari James
A. lindesayi ssp. *nilgiricus* Christophers
A. maculatus var. *willmorei* James
A. pallidus Theobald
A. ramsayi Covell
A. tessellatus Theobald
A. turkhudi Liston
A. annandalei Baini Prashad
A. culicifacies Giles
A. elegans James
A. gigas var. *baileyi* Edwards
A. hyrcanus nigerrimus Giles
A. jamesii Theobald
A. lindesayi Giles
A. maculatus Theobald
A. moghulensis Christophers
A. pulcherrimus Theobald
A. sintoni Puri
A. theobaldi Giles
A. umbrosus Theobald

Regarding *A. annandalei* Prashad, 1918, Dr. White states that the male and female syntypes of *A. djajasanensis* Brug, 1927 are available.

Regarding *A. jeyporiensis* James, 1902, it is stated that one male and four female syntypes of *jeyporensis* Theobald, 1903 (described from the same locality *viz.*, Jeypore State (now in Orissa, formerly in Madras State) are available. The original of James' types are not available.

Rijkamuseum Van Natuurlijke Historie, Leiden, Netherlands: Courtesy of Dr. P.J. Van Helsdingen: *Anopheles barbirostris* Van der Wulp. *Anopheles annularis* Van der Wulp. "Both are male and come from Mt. Ardjoeno to the south of Soerabaja in Java. The specimens were sold to the museum by Mr. Hekmeyer in 1872 as a part of a larger collection of insects". Both were marked 'Holotype' by Dr. Van Helsdingen.

Naturhistorisches Museum, Vienna, Austria: Courtesy of Dr. R. Lichtenberg: *Anopheles sinensis* Wiedemann

There are two paralectotypes in the Vienna Museum. The designation of paralectotypes was done by Dr. B.A. Harrison in 1973. They (a male and female) are in very poor condition.

According to Dr. Lichtenber (personal correspondence) a female lectotype also deposited by Dr. Harrison in the Universitites Zoologiske Museum Copenhangen, Denmark, is in excellent condition and bears two labels "Coll. Westerm." and '*Anopheles sinensis* Weid, China, Trentepohl". A female paralectotype in good condition is also deposited in the above museum.
(See also Harrison and Scanlon, 1973).

Museum National D'Histoire Naturelle, Paris, France: Courtesy of Dr. Loic Matile: Regarding the type of *Anopheles multicolor* Camboulin, stated to be in the Faculty of Medicine, Paris, is not there as it has no entomological museum. "Practically all old material of Insects of Medical importance can be considered as lost, until proof of the contrary, if it is stated to be deposited at the Faculte de Medicine. This is most probably the fate of the Camboulin Collection".

Zoologisches Museum, Museum der Naturkunde, Berlin, DDR: Courtesy of Dr. H. Schumann: The following types are in the above museum:

Anopheles leucosphyrus Donitz.
A. kochi Donitz
A. aconitus Donitz
A. vagus Donitz

U.S. National Museum of Natural History, Smithsonian Institution, Washington, U.S.A.: Courtesy of Dr. Wayne N. Mathis

The type of *Anopheles philippinensis* Ludlow is in the collection of the USNM (Type number 27783). It does not appear to be in the best of condition and is glued to a paper point.

For other Indian species, direct information is not available. The recent data provided by authors such as Knight and Stone (1977), Stone and Delfinado (1973) and Harrison and Scanlon (1975) have been adopted.

Appendix V

Conversion Tables

India has adopted the metric system, but all records previous to 1957 were made in the old foot/pound/second system. Inches, feet, yards and miles were used. Many references to dimensions in *Anopheles* literature have been made in that system. To directly convert them into centimetres, metres and kilometres often leads to expressing them in the new system with decimal numbers, which is not only inconvenient but gives a false accuracy. Therefore, while every effort has been made to express the old figures in the new system, still there are some occasions when the old terminology had to be used.

Given below is a short table showing the conversion figure which can be adopted where necessary.

1 inch	— 2.54 centimetres
1 foot	— 30.48 centimetres
1 yard	— 0.914 metres
1 metre	— 1.09 yards or 39.37 inches
1 mile	— 1.609 kilometres
1 kilometre	— 0.6214 miles
1 sq. metre	— 1.196 sq. yards
1 sq. inch	— 6.452 sq. centimetres
1 sq. foot	— 929.03 sq. centimetres
1 sq. yard	— 0.836 sq. metres
1 sq. mile	— 2.599 sq. kilometres
1 sq. kilometre	— 0.386 sq. miles
1 acre	— 0.405 hectares
1 hectare	— 2.471 acrs
1 gallon (imperial)	— 4.543 litres
1 litre	— 0.220 gallons (imperial)
1 ounce (weight)	— 28.38 grams
1 gram	— 0.035 ounces
1 pound	— 0.454 kilograms
1 kilogram	— 2.203 pounds

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INDEX

INDEX

In preparing this Index only selected items considered essential have been included and any item not included can be traced through the appropriate subject heading. While the names and subjects found in Part I are fairly well represented, those in Part II are repeated so often under almost every species that including them in the index would make it unweildy. The pages on which the individual species are dealt with in detail are indicated in bold type and the reader can refer to them for further location of any item.

Some names and items appear so frequently throughout the book that to list all of them would add to the length of the index without greatly adding to its value. Such items are indicated by the sign***. For most items only the pages on which they appear in a significant context are shown.

As an extensive list of references has been provided no separate author index has been considered essential. However names of persons which appear in the text without relation to any particular article have been indexed.

INDEX OF MOSQUITO NAMES

INDEX: GENERAL

ERRATA

Page	*Line*	*For*	*Read*
9	2	Neomyzomyia Series	Delete as it is duplicated
41	2	bering	bearing
44	16	*Cules*	*Culex*
61	3	Sun	sun
65	14	*Chara* southeren India	*Chara* in southern India
65	37	endmic	endemic
73	9	Mattingly (1976)	Mattingly (1967)
75	33	terms	term
86	28	dissimila	dissimilar
86	3 from below	Studies	studies
91	9	delete an	
110	26	markets	markers
110	32	insect	insects
119	5 from below	the latter	they
126	26	or	of
126	5 from below	projects	project
128	9	veritably	veritable
129	14	being made	were being made
130	30	tl	the
134	16 from below	and epidemic	and an epidemic
142	9 from below	have been	have not been
143	16	place	places
143	17 from below	delete etc.	
143	9 from below	purpose	purposes
147	22	development, tolerance	development of tolerance
149	15	Several	several
152	18	phenomena	phenomenon
153	4	unlike	As
155	28	several	reversal
161	15	reference	references
163	16	date	data
163	20	produce	produces
163	3 from below	if	of
185	21	groups	group
236	10	*cameronensia*	*cameronensis*
248	5	of	in
259	3	*P. malayi*	*B. malayi*
259	5	*P. malayi*	*B. malayi*
261	7	by	from
262	11 to 15	Upper bracket should in-clude figures for 18 to 19 hours.	
263	11 from below	Balaboc island	Balabac island
280	7	perdilection	predilection
281	5	belong	belongs
282	19	seasons	season
282	30	cannels	channels
293	18	40°C	24°C
301	16 from below	Introduce between "now" and *A. minimus—A. balabacensis* is the more important vector, though	
303	13 from below	cold water	cold weather
328	13	miles	mile
349	20	factors	factor
356	9	date	data

Page	Line	For	Read
357	14	Close of	Close to
361	11	long time	long time this species
362	3 from below	unconformed	unconfirmed
363	21	Aslamkhan and Salman (1967)	Aslamkhan and Salaman (1969)
365	25	Russell and Ramachandra Rao (1940 b)	Russell and Ramachandra Rao (1940 a)
365	last line	38, 362	35, 362
381	6	Delete "Additional and in the Andamans	
401	22	extensive in	extensive studies in
403	21	Delete "In Assam were equal"	
404	13 from below	feeds both on man and cattle	were found biting cattle.
407	20	vector filariasis	vector of filariasis
410	3	beds	pools
415	11	date	data
415	14	Insert close bracket after 115 females and delete bracket at the end of the sentence.	
415	16	some or	some of
418	19	water they grew	water in which they grew
423	14 from below	2.82	± 2.82
423	15 from below	440.00	440 to 585.00
423	16 from below	1.43	I 1.43
423	17 from below	130.00	130.00 to 225.00
432	9	scales or	scales of
433	22	Femore	Femora
438	2 from below	five	fine
439	22	appears	papers
449	26	acrs	acres
453	Asterisk (*) in references indicates that paper has not been seen in original by author.		
454	10	Merghai country	Menghai country
455	1	rodentenfeototion	rodent survey
455	3	BAISIS	BAISAS
456	23—25	BERTRA M, D.S. (1945)	Delete the whole reference
464	20	DE BURACA, B. (1946)	Delete the whole reference
467	21	*Malarial*	*Malariol*
472	2 from below	HALENNKAR	HARLENKAR
473	9	KRISHNASWAMY	KRISHNASWAMI
473	11	-do-	-do-
477	3 from below	KITXMILLERS, J. W.	KITZMILLER, J. B.
479	16 from below	house-flis	house flies
480	2 from below	POLCOOD, N.T. (1975)	POLEVOV, N.T. *et al.* (1975)
481	5	POLEVOV M.J. *et al.*	Delete whole reference as it is duplicated.
486	22	NAKVI	NAQVI
490	18	RAMATAHA RAO	RAMANATHA RAO
493	8 from below	efficiently	efficiency
496	2 from below	Teracolonization	Tericolonization
498	12	Rodent Walt	Roden Waldt
498	17	SUNDARESAN, B. AND RAO, M.A. (1945)	SUNDARESAN, B. AND RAO, M.A. (1943)
499	19	w	with
500	20	*Ventors*	*Vectors*
502	4	natural	nocturnal
502	15 from below	*Not. Soc.*	*Nat. Soc.*
502	7 & 8 from below	*Practical Molariology*	*Practical Malariology*
503	21	chomosterilized	chemosterilized